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HEREDITY IN HETEROGENEOUS HYBRIDS¹

JACQUES LOEB

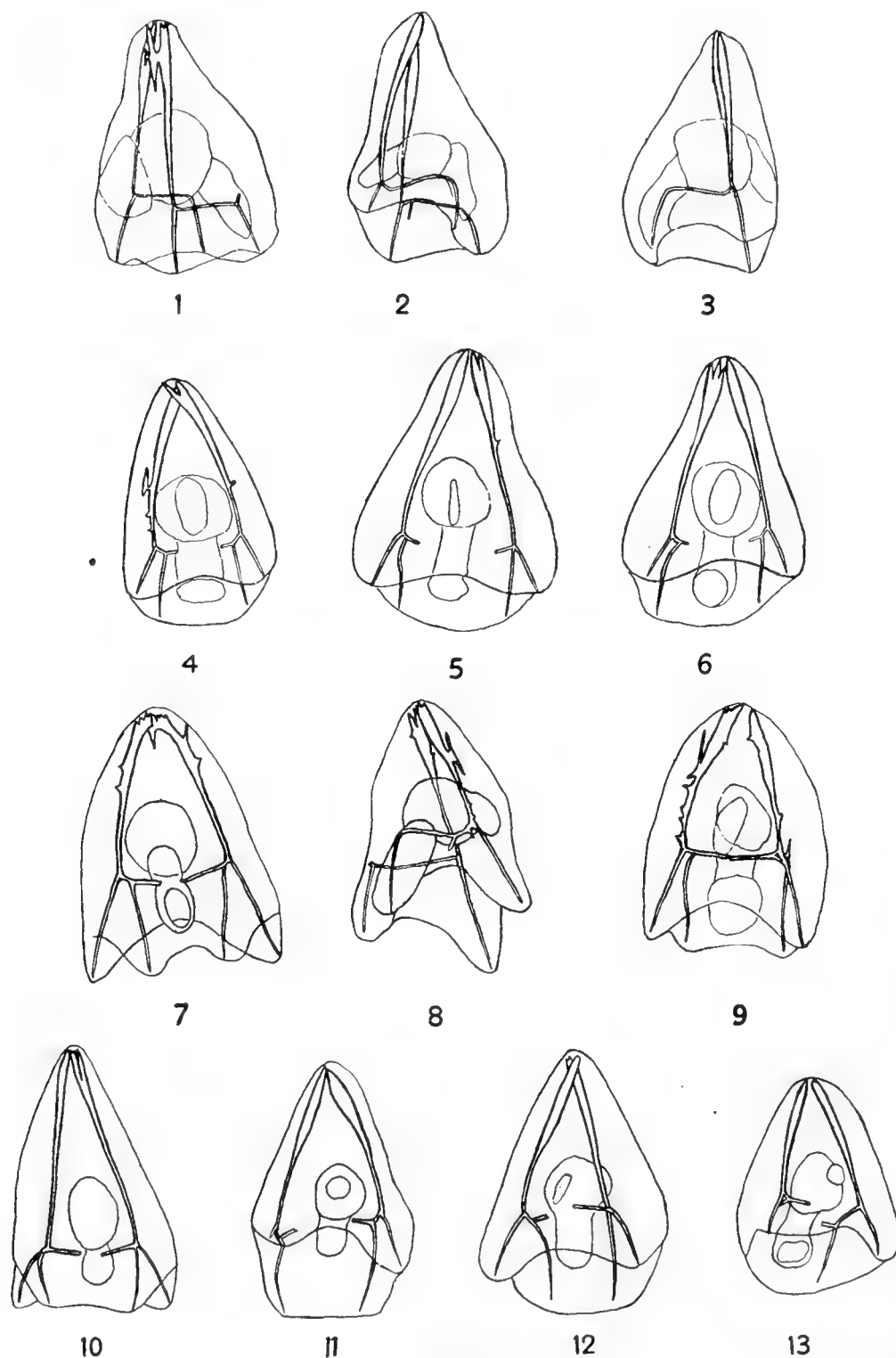
The Rockefeller Institute, New York

NINETEEN FIGURES

1. The study of heredity in embryos offers in one respect a wider field than that in adults inasmuch as heterogeneous hybrids rarely reach the adult stage. Eight years ago I found a method by which the eggs of the sea-urchin can be fertilized by the sperm of starfish, ophiurians and holothurians. The larvae are purely maternal, namely plutei. The results were confirmed by Godlewski for the fertilization of the egg of the sea-urchin by the sperm of the crinoid. It is well known that if we cross two homogeneous forms, e.g., two forms of sea-urchins, the paternal influence can be clearly seen in the pluteus stage. Since I have never published the figures of my experiments on heterogeneous hybridization, I may supplement my former statements with a few drawings. Figs. 1 to 6 are camera drawings of plutei of *Strongylocentrotus purpuratus* produced by artificial parthenogenesis. The plutei are, of course, in every detail identical with the plutei obtained if these eggs be fertilized with sperm of their own species. Figs. 7 to 9 are drawings of five days old plutei of *Strongylocentrotus purpuratus* ♀ and *Strongylocentrotus franciscanus* ♂. They differ from the pure breeds of *S. purpuratus* in several characters of the skeleton which exist in the pluteus of *franciscanus* but are absent from *purpuratus*, namely the greater roughness of the skeleton, the presence of cross bars and the greater length of the arms.² In figs. 10 to 13 are shown the five days old plutei of the egg of *S. purpuratus* fertilized with the sperm of the starfish (*Asterias*). It is obvious that the latter

¹ Prepared for the Whitman Memorial Volume but received too late to be included.

² Loeb, King and Moore, Arch. f. Entwicklungsmechanik, vol. 29, 1910.



Figs. 1 to 6 Five days old plutei of *Strongylocentrotus purpuratus* produced by artificial parthenogenesis.

Figs. 7 to 9. Five days old plutei of *Strongylocentrotus purpuratus* ♀ and *Strongylocentrotus franciscanus* ♂.

Fig. 10 to 13 Five days old plutei from *Strongylocentrotus purpuratus* ♀ and *Asterias* ♂.

plutei are purely maternal. It should, however, be borne in mind, that the objection might be raised that the presence of the skeleton in the sea-urchin pluteus might be dominant over its absence in the starfish larva. I have thus far vainly tried to produce a starfish larva with a pluteus skeleton by the hybridization of the two species.

We are therefore compelled to state that the hybrids between the sea-urchin egg and the starfish sperm represent more closely the purely maternal form than do the hybrids between two sea-urchins, which always show paternal characters.

2. It is well known that Herbst and Tennent have made experiments in which the paternal influence in the hybrid embryo was diminished. Tennent states that in the cross between *Hippoonoe* and *Toxopneustes*, *Hippoonoe* characters become dominant in sea water of a high OH concentration and *Toxopneustes* characters in sea water of a low OH concentration.³ The amount of acid or alkali Tennent needed to accomplish his result was very small; namely about 2 cc. $\frac{N}{10}$ acetic or hydrochloric acid to 500 cc. of sea water. A. R. Moore, W. R. King and myself made a number of experiments in which the hybrids between *S. purpuratus* and *S. franciscanus* were raised in sea water to which varying quantities of HCl or acetic acid or NaHO were added (from 0 to 0.4 cc. $\frac{N}{10}$ acid or NaHO to 50 cc. of sea water). We were able to retard or accelerate the rate of development, but the character of the hybrid remained absolutely unaltered.

I wish to call attention to the necessity of sterilizing the pipettes by boiling them after each experiment, instead of sterilizing them by rinsing in distilled or fresh water as is often done.

3. Moenkhaus measured the rate of segmentation in hybrid fish eggs and found that the rate for the first five cleavages is determined by the egg.⁴ The egg of *Ctenolabrus* segments about forty minutes after impregnation with sperm of its own kind, while the egg of *Batrachus tau*, if fertilized with the sperm of the same species, segments after about eight hours. If the egg of *Batrachus* be fertilized with the sperm of *Ctenolabrus* it also does not

³ Tennent, Publication 132, Carnegie Institution, 1910.

⁴ Moenkhaus, Am. Jour. of Anat., vol. 3, p. 29, 1904.

segment until after eight hours.⁵ I have repeated these experiments in a number of fish hybrids and confirmed Moenkhaus' results. The same facts are observed in the rate of development of hybrid embryos of echinoderms. What is the meaning of this fact? I believe that it finds its explanation through artificial parthenogenesis. These latter experiments have shown that the spermatozoon does not cause the development by carrying an enzyme or katalyzer into the egg, which the latter needs in order to develop, but causes the development by altering the surface layer of the egg. If the development of the egg were caused by an enzyme carried into the egg by a spermatozoon, the rate of cleavage of slowly developing eggs should be accelerated by a spermatozoon of a species developing at a faster rate. The egg however behaves exactly as we should expect from the fact that the spermatozoon removes only certain obstacles for the development of the egg but does not cause its development by carrying an activating enzyme. In order to cause the parthenogenetic development of the sea-urchin egg two different agencies are required and I have been able to show that the spermatozoon also causes the development of the sea-urchin egg by two agencies which act analogously to the agencies employed in my method of artificial parthenogenesis.⁶ F. Lillie has found the same in *Nereis*.⁷ It was therefore to be expected that the rate of development of embryos was determined by the egg. This view meets with a difficulty in the fact that, with a few exceptions, the later development of hybrids is as a rule retarded. But this difficulty is only an apparent one since the retardation is due to an entirely secondary condition: namely that most hybrids are sickly and not able to hatch or reach an adult state.

This is most strikingly the case in the heterogeneous hybrid, e.g., in the cross between the sea-urchin and the starfish. As I pointed out long ago the larvae die mostly in the gastrula stage,

⁵ The acceleration of segmentation which Newman observed in the egg of *Fundulus majalis* fertilized by the sperm of *Fundulus heteroclitus* is too small to influence our conclusions.

⁶ Loeb, *Die chemische Entwicklungserregung des thierisches Eies*. Berlin, 1909, and *Das Wesen der formativen Reizung*, Berlin, 1909.

⁷ F. R. Lillie, *Jour. of Morph.*, vol. 22, 1911.

and possibly one egg in a million reaches the pluteus stage. The development of the pluteus is in such cases always retarded.

We find such a retardation not only in the case of heterogeneous hybridization but occasionally also in the case of crosses between closely related forms. While the hybrid *purpuratus* ♀, *franciscanus* ♂ is vigorous, the hybrid *franciscanus* ♀, *purpuratus* ♂, is sickly and reaches the pluteus stage only rarely and slowly.

The rate of development of the embryo is a function of the velocity of certain chemical processes which are linked together like the wheels in a mechanical machine. If one of such processes be retarded the rate of development of the whole embryo is likely to be retarded; and if the linkage of the various chemical processes become disturbed the embryo is likely to be sickly. The further apart the species are from which the two sex cells originate the greater the likelihood that the rate of development is retarded and that the hybrid embryo is sickly. Why is the development not retarded from the beginning? Possibly for the reason that it requires some time before the spermatozoon can cause the formation of a sufficient amount of harmful substances to cause a retardation of the development of the egg.

4. Moenkhaus found that the eggs of bony fishes can be easily impregnated with foreign sperm but that they do not develop very far. Thus he states, that the hybrids between *Menidia* and *Fundulus heteroclitus* "never go beyond the closure of the blastopore." I have been able to raise the hybrid between *Fundulus heteroclitus* ♀ and *Menidia*, *Ctenolabrus* and *Stenotomus* ♂ in large numbers beyond this stage. These hybrids lived a month or longer, formed hearts, blood vessels, eyes, and fins but never hatched. With a few exceptions no circulation was ever established although the heart beat for weeks.

Figs. 14 to 16 show three different hybrids of *Fundulus heteroclitus* ♀ and *Menidia* ♂, from a lot fertilized the 12th of June. The camera drawings were made from the living material the 2d, 3d and 12th of July. At that time the pure breed of *Fundulus heteroclitus* fertilized on the same date were already hatching. The hybrid embryos had formed the pigment characteristic for the pure breed of *Fundulus heteroclitus*. But the anomalies of

the embryos are very obvious. The embryos are rather small, owing to the slowness with which they digest the yolk. Their eyes are abnormal and approach the cyclopean condition. In many specimens only irregular masses of pigment indicate where the eyes should be. The head is comparatively small and not bent as is characteristic for the pure breed. The heart is developed but corresponds to an early stage in the development. It beats regularly and at an almost normal rate. The main blood vessels exist and haemoglobin is formed but the creeping of the pigment cells upon the blood vessels does not take place.

Years ago I found that the marking of the yolk sac of *Fundulus* and of the embryo is caused by the creeping of the chromatophores upon the blood vessels. I showed that this phenomenon is due to a tropism which depends upon the circulation. When the circulation was suppressed pigment was developed but the chromatophores did not creep upon the blood vessels. At that time I had succeeded in suppressing the circulation for a few days.⁸ In the new experiments the hybrid embryos lived for a month or more with pigment but without a circulation. They demonstrate the correctness of my former statement, inasmuch as the creeping of the chromatophores upon the blood vessels did not take place. They also confirm the statement, that the formation of pigment cells is independent of the circulation. Newman seems to hold the opposite view, but he evidently did not test his assertion experimentally.

These hybrids were also smaller than the pure breeds of the same age, owing to the fact that the yolk is less rapidly digested in the hybrids than in the pure breeds. This is a very important link in our conclusions on heredity. The development of hereditary characters is the result of the nature and the velocity of chemical reactions between the mass of yolk on the one hand and the substances in the nucleus, especially the chromosomes, on the other. If two closely related forms be crossed, the chemical reactions are not materially different in quality and velocity from those of the pure breeds. But when distant forms are crossed it is to be expected that greater differences in the nature and the

⁸ Loeb, *Jour. of Morph.*, vol. 8, 1893; Woods Hole Lectures.

rate of chemical reactions will be found and the outcome will be pathological embryos and very likely a suppression of the paternal influence. The disturbance is the same in practically all the heterogeneous hybrids. I have also produced the crosses between *Ctenolabrus* ♂ and *Fundulus heteroclitus* ♀ and between *Stenotomus* ♂ and *Fundulus heteroclitus* ♀. In all cases the result was about similar to the one described here. In all cases there was a consumption of yolk, development of an embryo, of pigment, of a heart beat, of eyes, lenses, ears, fins; but, with rare exceptions about which we are to speak later, there was no circulation. The number of relatively good embryos was very large in the cross between *Fundulus heteroclitus* ♀ and *Menidia* ♂ (where about 90 per cent formed embryos that lived for about a month); it was much smaller in the cross between *Fundulus heteroclitus* ♀ with *Ctenolabrus* ♂. One word should be said in regard to the development of the head in these embryos. In later stages it is often abnormally small in comparison with the body. The reason for this is that, although at first the head of these heterogeneous hybrids develops normally, sooner or later its development stops and often phenomena of degeneration set in, especially in the eyes. The body of such larvae however continues to grow.

5. From what was said before, I reached the conclusion, that these hybrid larvae between *Fundulus* ♀ and *Menidia* ♂ were in reality pure breeds, namely *Fundulus heteroclitus* larvae whose development was retarded through some interference with the normal chemical reactions in the egg; and that the abnormalities described were in no way hybrid characters. It occurred to me that it might be possible to produce similar larvae from pure breeds of *heteroclitus* eggs, if the latter were compelled to develop under an abnormal chemical condition. For this purpose the following experiment was made. Eggs of *Fundulus heteroclitus* were put immediately after fertilization into closed Erlenmayer flasks, each of which contained 50 cc. of sea water to which various amounts of a 0.01 per cent solution of NaCN were added, from 0 to 10 cc. The eggs remained in these solutions about a month. In the mixture of 2 cc. 0.01 per cent NaCN and 50 cc. of sea water, embryos were found which in every way resembled the hybrids

between *Menidia* ♂ and *Fundulus heteroclitus* ♀. (See figs. 17 and 18.) Their eyes were poorly developed, they had a tendency to form cyclopean eyes, the yolk was incompletely digested and the embryo too small. The heart was formed and beat, but no circulation was established. Pigment was formed (in the drawing most of the black pigment cells on the yolk sac were not indicated, since the drawing was intended for another purpose). The head is not bent against the body, and so on. In all respects these sickly embryos of pure breed resemble the hybrids between *Fundulus heteroclitus* ♀ and *Menidia* ♂.

Since NaCN acts through a retardation in the rate of oxidation, the idea might be expressed, that in heterogeneous hybrids oxidations are interfered with. It is not safe to accept such an idea until it has been tested experimentally.

6. The idea that the heterogeneous hybrids in fish are purely or at least essentially maternal finds support in the fact that a small number of these hybrids develop more normally than those thus far mentioned. In a small number of such hybrids circulation is established, though as a rule rather late and often for a few days only. But these embryos develop rather normally and are, as far as any present observations go, purely maternal. I have had many such hybrids but I will give only one camera drawing (fig. 19), representing a twenty-five day old hybrid between a male scup and a female *Fundulus heteroclitus*. The yolk is in this case pretty well digested.

7. While it is the rule that in the case of heterogeneous hybridization heredity is purely maternal, it is possibly not without exception. I have however thus far found only one paternal character which is possibly transmitted to a heterogeneous hybrid. The yolk sac of the egg of *Fundulus heteroclitus* forms branched red chromatophores which are not found on the yolk sac of *Menidia*. In two eggs of *Menidia* fertilized by sperm of *Fundulus heteroclitus* a few red chromatophores were observed. It is difficult to get this cross and I give this observation with some hesitancy.

8. Kupelwieser and Baltzer account for the fact that heterogeneous hybrids are purely maternal by the assumption that the

chromosomes of the sperm are thrown out of the egg or disintegrate. This is not in harmony with the observations of Moenkhaus for the hybrids between *Menidia* ♂ and *Fundulus heteroclitus* ♀, and with those of Godlewski for the hybrids between sea-urchin and crinoid. I am not in a position to decide the differences in the observations of these authors. The observations mentioned in the preceding paragraph are more in harmony with the observations of Moenkhaus and Godlewski.

CONCLUSIONS

The spermatozoon has two distinct effects upon the egg: namely, it causes its development and it transmits certain parental hereditary characters to the offspring. The experiments in heterogeneous hybridization confirm the idea supported by the experiments on artificial parthenogenesis, that the formation of the embryo is purely a matter of the egg and that the main function of the spermatozoon is the causation of the development of the egg. If we may express this statement in the form of a paradox we may say that fertilization is primarily and essentially artificial parthenogenesis. The transmission of hereditary characters through the sperm is in many cases merely an accessory function. It becomes of vital importance only in those forms where the male is heterozygous for sex and where the species can only be propagated through sexual reproduction.

If the sperm nucleus be chemically almost identical with the egg nucleus it is possible for it to force one or a few characters upon the developing embryo. If the difference between sperm and egg nucleus exceed a certain limit—which structural chemistry may one day be able to define—the hereditary influence of the spermatozoon is as a rule completely or almost completely obliterated; and the result is a purely maternal larva, rendered more or less sickly through the presence or formation of foreign or faulty substances.

The camera drawings of the sea-urchin larvae were made by Mr. W. O. R. King, those of the fish embryos by Mr. Bagg. To both gentlemen I wish to express my thanks.

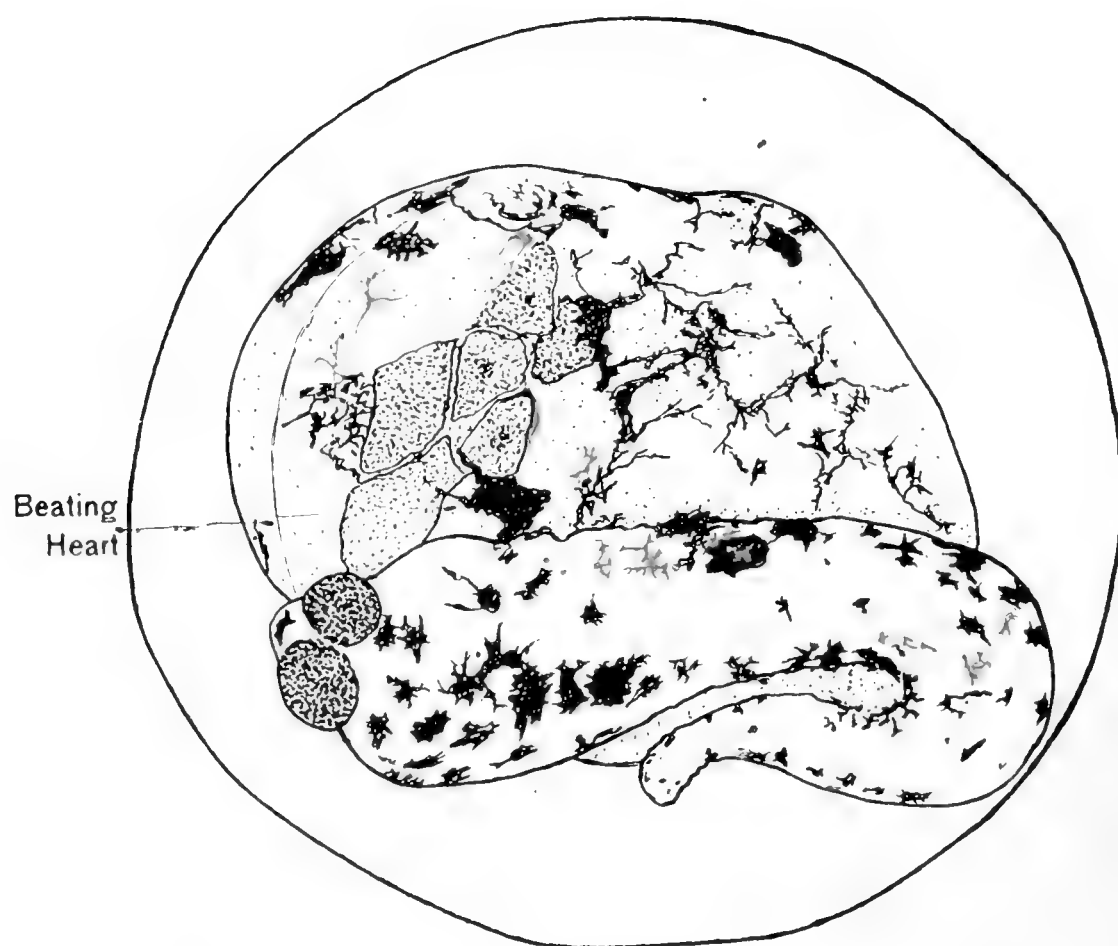


Fig. 14 Hybrid between *Fundulus heteroclitus* ♀ and *Menidia* ♂, three weeks old.

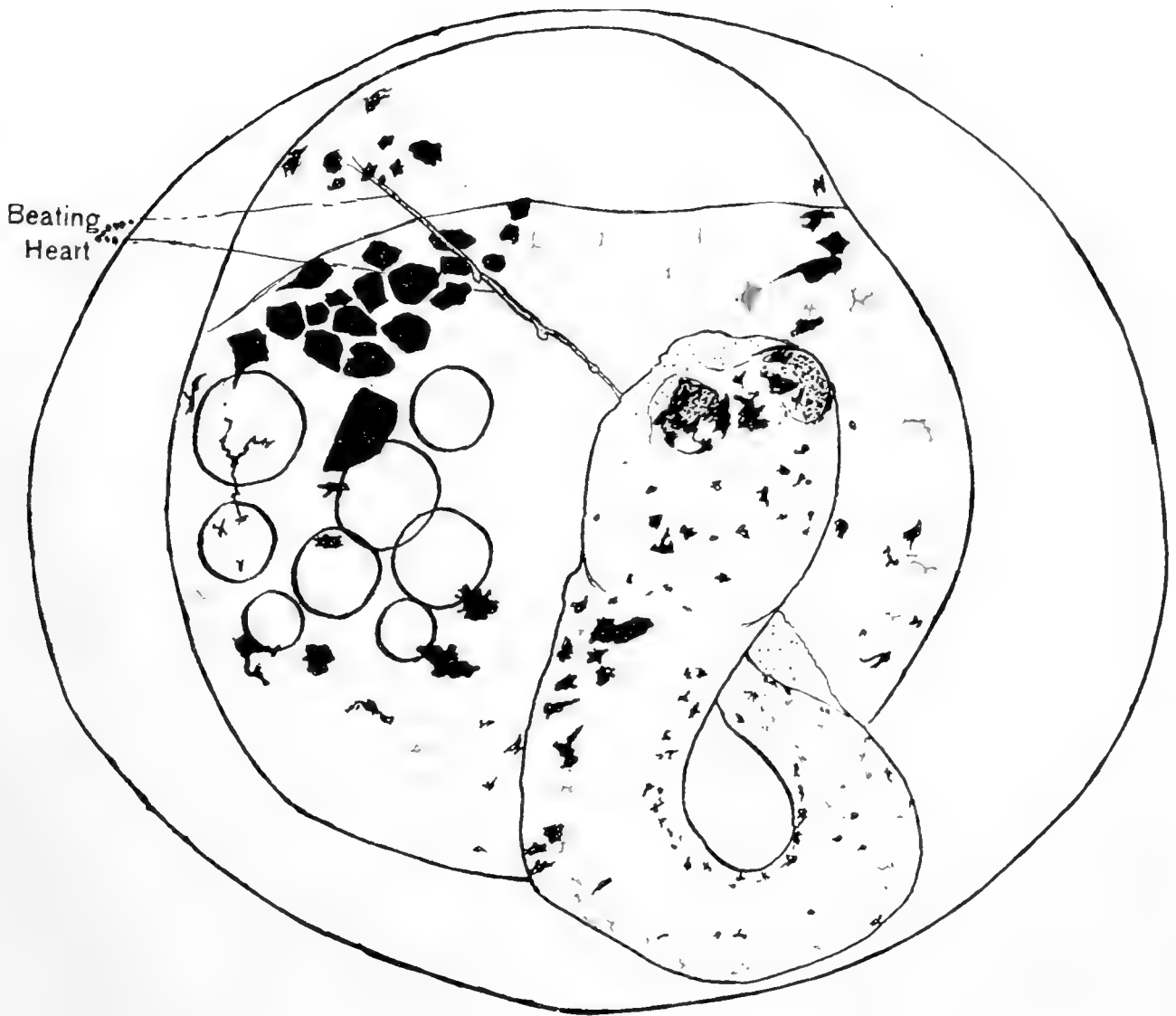


Fig. 15 Hybrid between *Fundulus heteroclitus* ♀ and *Menidia* ♂, twenty days old.

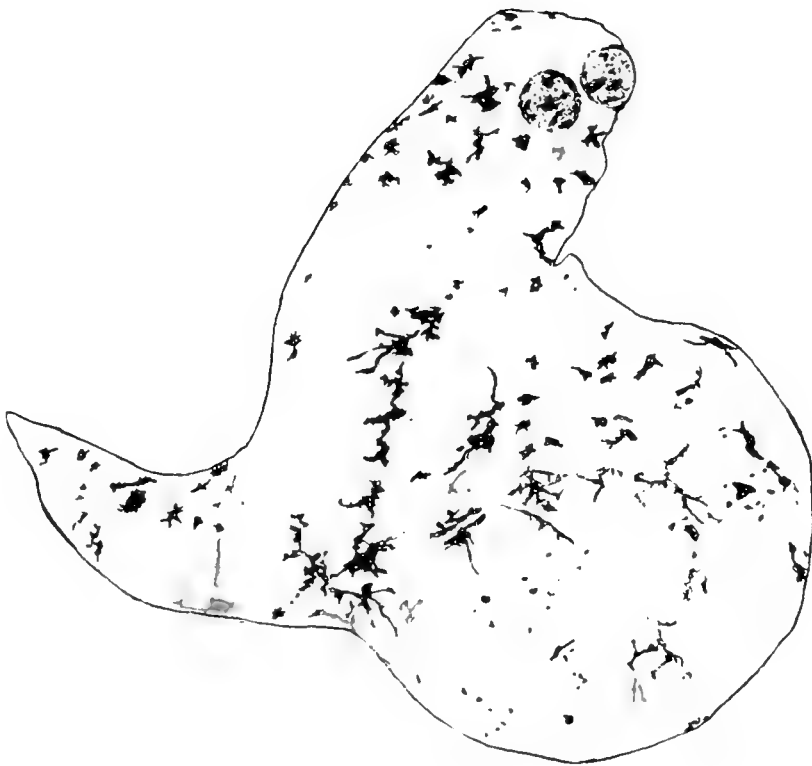


Fig. 16 Hybrid between *Fundulus heteroclitus* ♀ and *Menidia* ♂, thirty days old.

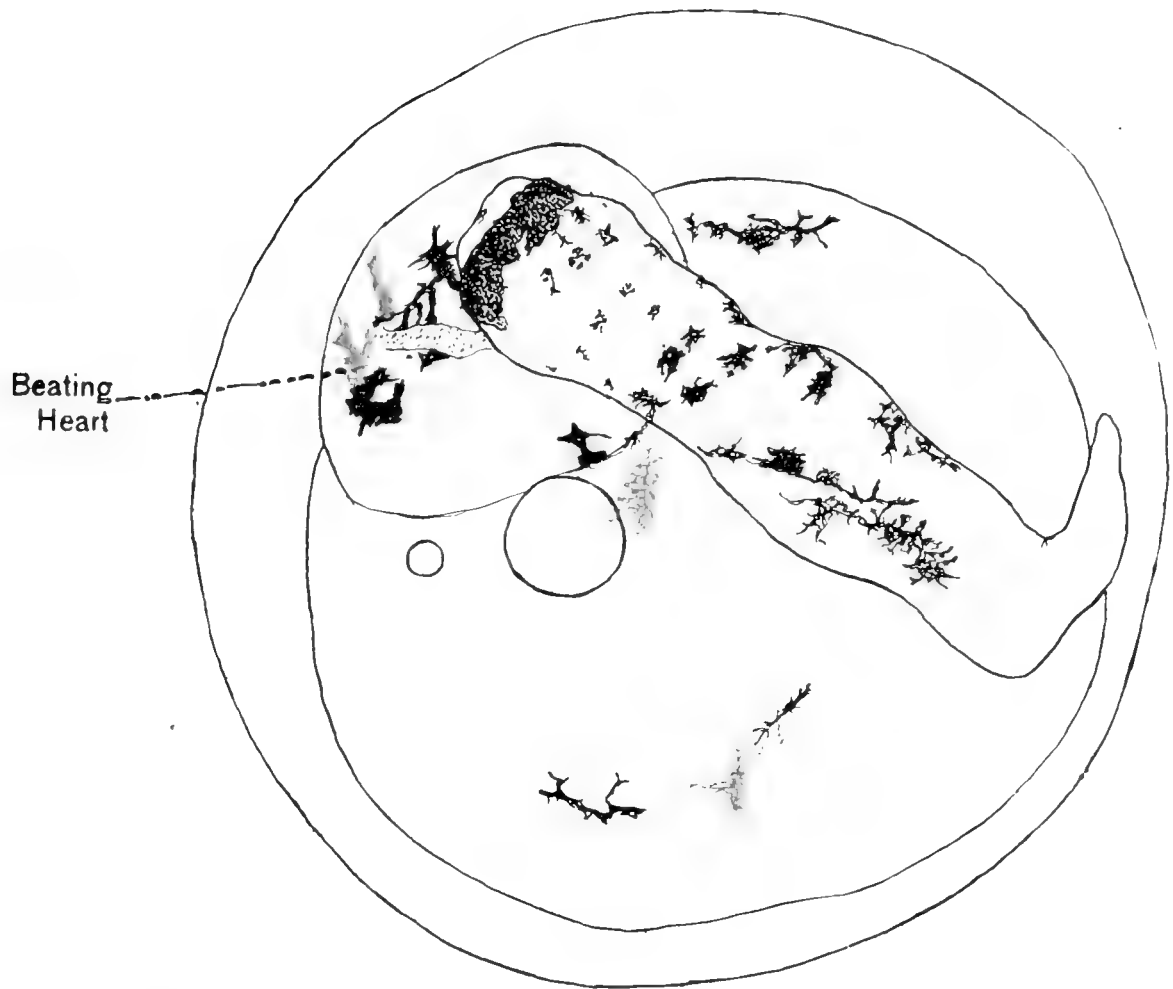


Fig. 17 Embryo of *Fundulus heteroclitus* thirty-one days old, raised in 50 cc. sea water + 2 cc. 0.01 per cent NaCN.

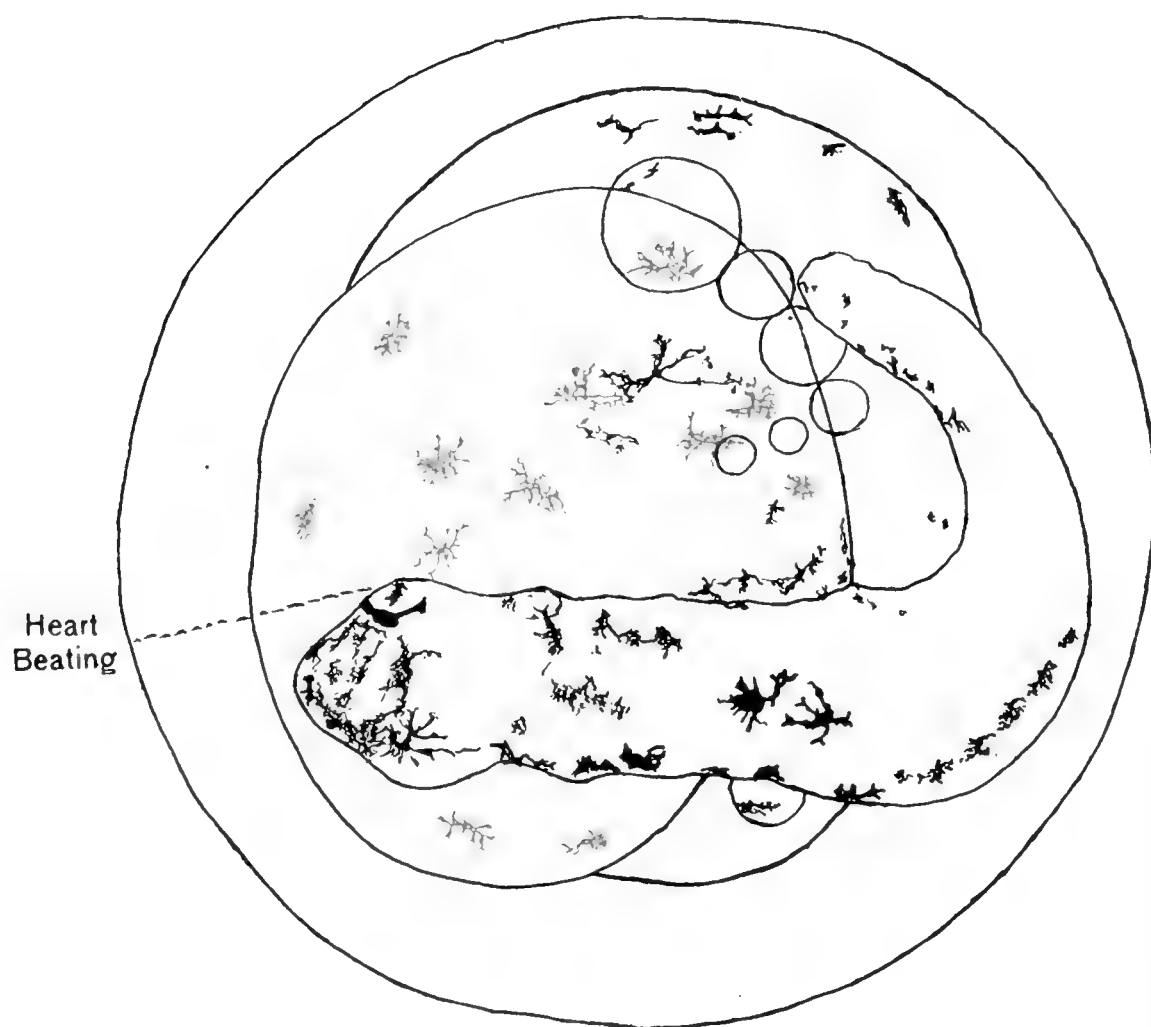


Fig. 18 Embryo of *Fundulus heteroclitus* thirty-one days old, raised in 50 cc. sea water + 2 cc. 0.01 per cent NaCN.

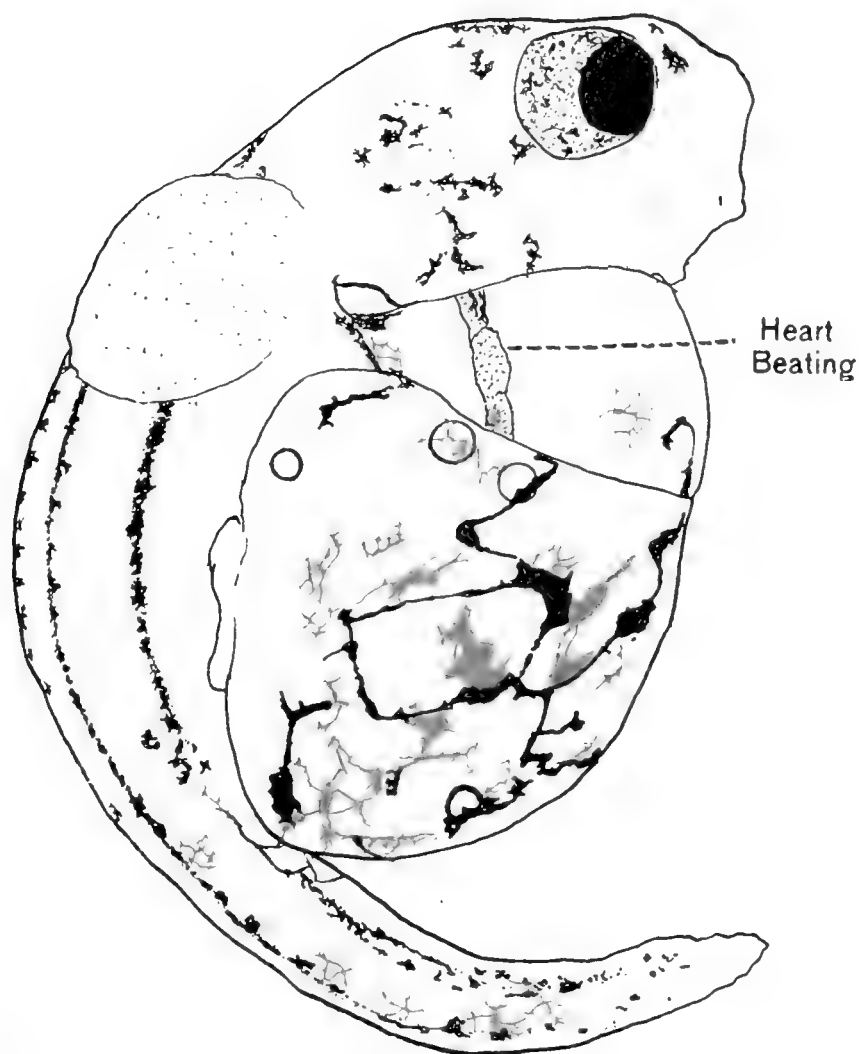


Fig. 19 Hybrid between *Fundulus heteroclitus* ♀ and scup ♂, twenty-five days old.

THE BEHAVIOR OF THE CHROMOSOMES IN CROSS FERTILIZED ECHINOID EGGS¹

DAVID H. TENNENT

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TWENTY FIGURES IN TWO PLATES

It is my purpose to present in this paper some of the facts gained by a cytological study of Echinoid crosses and to consider critically the results of Baltzer and Herbst in similar studies.

In my first work on the chromosomes of cross-fertilized Echinoid eggs ('08) I found that beyond the mere determination of species differences in chromosomes, little could be done until a careful study of the chromosomes of the parent forms, in all phases of mitosis, had been made.

Since that time important investigations on the straight-fertilized eggs of some of the parent species have been completed by two workers in my laboratory, Dr. Heffner ('10) and Miss Pinney ('11) so that we are now prepared to identify somewhat adequately the chromosomes in *Toxopneustes* × *Hipponoë*, *Toxopneustes* × *Moira*, *Arbacia* × *Moira* and *Arbacia* × *Toxopneustes* crosses. In addition we now have the important studies of Baltzer ('09, '10) and Herbst ('09) on European forms, for comparison.

During the past year I have investigated reciprocal *Toxopneustes* × *Hipponoë* crosses made in normal sea water and the same crosses made in sea water whose alkalinity had been reduced as described in my papers of 1910–1911.

Heffner ('10) showed for *Toxopneustes* long rods and two Vs or three Vs. Pinney ('11) showed for *Hipponoë* four Vs in all eggs and in addition a long armed V, or hook shaped chromosome, in half of the eggs. My own observations on the *Toxopneustes*

¹ Prepared for The Whitman Memorial Volume, but received too late to be included.

♀ × Hipponoë ♂ cross ('11) showed that a hook-shaped chromosome was present in 50 per cent of the eggs and that it must have had its origin in the paternal, i.e., Hipponoë, nucleus. I shall proceed immediately to a consideration of the crosses.

TOXOPNEUSTES ♀ × HIPPONOË ♂

As a general comparison between the chromosomes of straight-fertilized *Toxopneustes* eggs and those of straight-fertilized *Hipponoë* eggs, it may be stated that in *Toxopneustes* the chromosomes are more slender and less closely massed on the spindle than in *Hipponoë*. The massing of *Hipponoë* chromosomes is due, in part, to their larger size and the fact that the *Hipponoë* egg and amphiaster are smaller than those of *Toxopneustes*. But in spite of the fact that the *Hipponoë* chromosomes are larger, when studied in straight-fertilized eggs, I have found it impossible to distinguish in general between the chromosomes of the two species in cross-fertilized eggs; that is, I cannot find one group of slender chromosomes and another group of thicker chromosomes, which I can identify as of *Toxopneustes* and *Hipponoë* respectively. In the cross-fertilized eggs all seem to be of about equal thickness.

The chromosomes of the straight-fertilized eggs are in general rod-like in form, some long, some short; in particular, a few have individual peculiarity of form. These latter in *Toxopneustes* (Heffner, '10) are either two Vs or three Vs and two long rods; in *Hipponoë* (Pinney, '11) four Vs, or four Vs and a hook-shaped chromosome.

When we examine the mitotic figures of *Toxopneustes* ♀ × *Hipponoë* ♂ material (figs. 1-11) we may identify these elements readily. In fig. 1, four Vs, a long rod, a hook and, in addition, two other bent elements (fig. 1, C) which I have never found in other division figures. Fig. 2, three Vs and a hook. Fig. 3, three Vs and a hook. Fig. 4 three Vs and a hook. Fig. 5 three Vs a long rod and no hook. Fig. 6, two Vs in early anaphase. Fig. 7, two Vs and no hook. Fig. 8, three Vs and no hook. Fig. 9, three Vs and no hook. Fig. 10, two Vs. Fig. 11, three Vs and a hook.

In a careful study of fifty-two amphiasters in which all of the elements might be seen with clearness sufficient to enable me to determine the presence or absence of the hook-shaped chromosome, it was found to be present in twenty-eight instances and not present in twenty-four, from which I have concluded that it occurs in half of the *Toxopneustes* eggs which have been fertilized with *Hipponoë* sperm.

The point which I wish to emphasize here is that *an unpaired element seen in straight-fertilized Hipponoë eggs is thus shown to be carried by the Hipponoë sperm.*

HIPPONOË ♀ × TOXOPNEUSTES ♂

If we turn now to the figures of the reciprocal cross (figs. 12 to 20), we will find that in all instances three Vs are present while *a hook-shaped chromosome is never found.* The identification of the Vs is sometimes made with difficulty since the arms of the V may be brought closely together during anaphase, the result being that the V acquires the appearance of a very thick rod. In figs. 18 and 20 the Vs may be seen with separated arms; in the other figures one or more of the Vs are seen with the arms in contact.

CONSIDERATION OF THE CROSSES

This cross-fertilization has given us a conclusive means of solving the problem of the origin of the hook-shaped chromosome in *Hipponoë*. Let me restate our facts.

1. The hook-shaped chromosome is found in half of the straight-fertilized *Hipponoë* eggs. It is an unpaired element. From a study of these eggs we have no means of determining whether it is an element peculiar to the egg or to the sperm nucleus.

2. The hook-shaped chromosome is found in one half of the *Toxopneustes* eggs which have been fertilized by *Hipponoë* sperm. An element of this form does not occur in *Toxopneustes*, therefore it has been brought in by the *Hipponoë* spermatozoan and occurs in half of the spermatozoa.

3. The hook-shaped chromosome is not present in the *Hipponoë* ♀ × *Toxopneustes* ♂ cross. This indicates that it is not present

in the Hipponoë egg and furnishes corroborative evidence that our conclusion reached in the second statement is correct.

Our analysis has given conclusive evidence that in Hipponoë there is a heterochromosome which is of paternal origin. This evidence is of value since it indicates that the conclusion drawn from Baltzer's ('09) investigation, which is supported by the work of Heffner ('10), i.e., that in Echinoids the female is the heterogametic sex, while the male is homogametic, is not of general application, and that in one Echinoid at least, Hipponoë, we have conditions which are similar to those found in insects.

THE BEHAVIOR OF THE CHROMOSOMES IN CROSS-FERTILIZED ECHINOID EGGS

A very different phase of my work is concerned with the idea of the fate of the chromosomes in these crosses and the correlation of this behavior with the environment.

Baltzer ('09, '10) and Herbst ('09) have shown the fact of chromosome elimination in various crosses. Baltzer ('10, pp. 608-609) has given a valuable tabular summary of facts in Echinoid crosses, with the character of the resulting pluteus. In most instances chromosome elimination is followed by a maternal pluteus and chromosome retention is followed by an intermediate pluteus. In two crosses, however, *Echinus* ♀ × *Antedon* ♂ and *Strongylocentrotus* ♀ × *Antedon* ♂, there is no elimination, and the skeleton of the pluteus is maternal in character.

In other crosses, *Strongylocentrotus* ♀ × *Echinus* ♂ and *Sphaerechinus* ♀ × *Arbacia* ♂, there is no elimination and a 'maternal intermediate' pluteus results.

For the crosses under consideration in this paper I have previously shown ('10, '11) that the plutei resulting from the cross, no matter in which direction the cross has been made, have a skeleton which resembles that of the Hipponoë pluteus more nearly than it does that of *Toxopneustes*, and I have stated that as a result of this cross we have a Hipponoë dominance with respect to the character of the skeleton. This dominance is not complete and in many instances the skeleton is of a 'Hipponoë intermediate' type.

We may get at the matter before us most quickly by seeking to ascertain whether in either or both of the *Toxopneustes* \times *Hipponoë* crosses there is an elimination of chromosomes.

I am not able at this time to make a final statement as to the exact number of chromosomes in either *Toxopneustes* or *Hipponoë*. In *Toxopneustes* it is either 36, 37 or 38. In *Hipponoë* 32, 33 or 34. This is sufficiently near to enable us to determine a noticeable elimination. In my illustrations the numbers found (and preparations were selected in which I believe that I was able to count all of the chromosomes that were present) were fig. 1, upper 34; lower 34; fig. 2, 30 and 31; fig. 3, 34 and 32; fig. 4, 30 and 31; fig. 5, 33 and 33; fig. 7, 33 and 34; fig. 8, 38 (?) and 35 (?).

Similar counts hold for older segmentation stages up to the 32 to 64 cell stages, so that we have in this cross no chromosome elimination. The *Hipponoë* chromosomes are retained and we have in correlation *Hipponoë* dominance.

With the reciprocal cross, *Hipponoë* $\varnothing \times$ *Toxopneustes* σ the counts range; fig. 12, 22 and 29; fig. 13, 30 and 36; fig. 14, 28 and 24; fig. 15, 27 and 24; fig. 18, 31 and 32 (those at center not counted); fig. 17, 30 and 25 (anaphase four cell stage, one at center not counted); fig. 20, sixteen cell stage, 16 and 16 (two at center not counted).

It will be seen from these examples that in this cross the behavior of the chromosomes is irregular from the beginning and that in some instances there is an elimination of fully half of the chromosomes, presumably *Toxopneustes* chromosomes, by the time the sixteen cell stage has been reached.

The facts regarding the chromosomes and the correlation with dominance in these crosses may be stated in general terms, *Toxopneustes* $\varnothing \times$ *Hipponoë* σ

Without chromosome elimination, *Hipponoë* dominance.
Hipponoë $\varnothing \times$ *Toxopneustes* σ

With chromosome elimination, *Hipponoë* dominance.

In the *Hipponoë* $\varnothing \times$ *Toxopneustes* σ cross there is a constant elimination of chromosomes up to the sixteen and immediately succeeding cell stages until only (?) *Hipponoë* chromosomes remain. This elimination is brought about by lagging and failure

to be included in the reconstructed daughter nuclei. In fig. 13, *A* and *B*, and fig. 14, *A* and *B*, many chromosomes which are lagging in the first division may be seen. In fig. 17, *A* and *B*, a continuation of this elimination by a similar process during the third division is shown.

A further part of my investigation is concerned with the study of the eggs fertilized in sea water of decreased alkalinity. Unfortunately I did not obtain eggs in sufficiently late stages of segmentation to enable me to give a final statement of the results. Figs. 9 and 10 are representative of the anaphases of the first division in *Toxopneustes* eggs fertilized by *Hipponoë* sperm in sea water whose alkalinity had been reduced by the addition of acetic acid.

In all of this material more lagging chromosomes are found and the average number of chromosomes present in the daughter plates is smaller than in eggs fertilized in normal sea water.

I had hoped to show that a behavior of the chromosomes correlated with the results of the artificial control of dominance might be demonstrated. Such a correlation would be indicated by the elimination of *Hipponoë* chromosomes in the *Toxopneustes* ♀ × *Hipponoë* ♂ cross.

As the figures show, there is some evidence that such an elimination takes place but the evidence is not sufficient.

The study of late segmentation stages should determine the question at once.

DISCUSSION AND COMPARISON WITH RESULTS OF OTHER INVESTIGATORS

The time has not yet come when we may give a satisfactory discussion of the meaning of our facts and make a trustworthy correlation of these with those determined for insects.

Herbst's ('90) work was valuable in showing the elimination of chromosomes, or rather the failure of the paternal chromosomes to take part in the activities of mitosis. But Herbst's experiments do not show that a changed environment results in a change in the character of the pluteus. Chemical fertilization would have given, as Herbst shows, maternal plutei. Delayed fertilization of these chemically treated eggs gives plutei of the

maternal type simply because the male nucleus has entered too late to take a normal part in the process. A partial union may give rise to a partially thelykaryotic pluteus. In some cases an apparently complete union with a subsequent elimination of *Strongylocentrotus* chromatin occurred. Baltzer ('10) in his study of the same cross, found that there was no elimination of chromosomes under normal conditions, and that plutei with an intermediate type of skeleton were found.

All that can be claimed for the result of Herbst's treatment is that the sperm was added so late that, when the modified fusion of the pronuclei did take place, the effects of the chemical fertilization had attained too great an impetus to be overcome by the materials of the paternal nucleus. He obtained varying degrees of modification. All were not modified; in all, the result depended on the amount of development that had been attained in the thelykaryotic activities.

This is precisely the result that I obtained with the starfish egg ('06) by the superposition of fertilization on artificial parthenogenesis. If the sperm were added before the egg nucleus had gone too far, a union of the pronuclei and a normal division followed. If the addition were made later, the paternal chromatin entered the mitosis irregularly and was in part rejected.

Baltzer's important results lie in his accumulation of facts regarding individual chromosomes and in his determination of elimination and non elimination.

I have shown that his idea of the female Echinoid as the heterogametic sex must be restricted to the cases in which it has been observed and cannot be used as a general interpretation.

I realize the impossibility of interpreting the results of another investigator from drawings, without having seen the sections from which the illustrations were made. It is therefore with hesitation that I suggest that Baltzer's fig. 23a ('10) shows for the *Sphaerechinus* ♀ × *Strongylocentrotus* ♂ cross exactly what I have shown for *Toxopneustes* ♀ × *Hipponoë* ♂.

Baltzer ('09) has shown that the particular chromosomes of *Strongylocentrotus* are two long hooks or two long hooks and a short hook. (See Baltzer, '09, figs. 1a, 1b, 5a, 5b and 11, 12 a-d.)

The short hook is definitely localized, according to Baltzer, in the egg (text and diagrams, p. 579). The fact that it does not occur in his figs. 16*a* and *b* nor in 17*a*, *b* and *c* harmonizes with this idea; but when we turn to fig. 23*a* (Baltzer, '10)—*Sphaerechinus* ♀ × *Strongylocentrotus* ♂—we find what might be interpreted from the illustration as a short hook. The author definitely states ('10, p. 509), that he has never found a hook-shaped chromosome in *Sphaerechinus*. If both of these apparent hooks be such, one of them must be the unpaired element seen in half of the straight-fertilized *Strongylocentrotus* eggs. It is also difficult to understand why the elongated rods in the anaphase plates (Baltzer's metaphase) in figs. 6*b*, 7*b* and 11 ('09) should not be regarded as long rods, as in *Echinus* (Baltzer 2*a* and *b*, 3*a* and *b*). My hesitation in speaking of these possible interpretations is overcome only by the fact that the conditions in the illustrations are similar to division figures in my own material.

The investigations of Heffner ('10) on *Toxopneustes* show that this is in some respects like *Strongylocentrotus*. Heffner shows for straight fertilized *Toxopneustes* eggs two Vs three Vs and two long rods. The Vs may be regarded as comparable to the hooks. The long rods in *Toxopneustes* are like the long rods shown in Baltzer's figures of *Strongylocentrotus*. We need facts concerning the chromosomes in many species of Echinoids. They should be studied not only in straight-fertilized eggs, but in crosses, in chemically fertilized material and in fertilized enucleated egg fragments.

LITERATURE CITED

- BALTZER, F. 1909 Die Chromosomen von *Strongylocentrotus lividus* und *Echinus microtuberculatus*. Arch. f. Zellforsch., bd. 2.
- 1910 Über die Beziehung zwischen dem Chromatin und der Entwicklung. Arch. f. Zellforsch., bd. 5.
- HEFFNER, B. 1910 A study of chromosomes of *Toxopneustes variegatus* which show individual peculiarities of form. Biol. Bull., vol. 19.
- HERBST, C. 1909 Vererbungsstudien VI. Arch. Entwicklungsmech. bd. 27.
- PINNEY, M. E. 1911 A study of the chromosomes of *Hipponoë esculenta*. Biol. Bull., vol. 21.
- TENNENT AND HOGUE 1906 Studies on the development of the starfish egg. Jour. Exp. Zool., vol. 3.
- TENNENT, D. H. 1908 The chromosomes in cross-fertilized echinoid eggs. Biol. Bull., vol. 15.
- 1910 Dominance of maternal or of paternal characters in echinoderm hybrids. Arch. f. Entwicklungsmech., vol. 29.
- 1911a Echinoderm hybridization. Carnegie Institution pub. 132.
- 1911b A heterochromosome of male origin in echinoids. Biol. Bull., vol. 21.

PLATE 1

EXPLANATION OF FIGURES

All figures are drawn to a magnification of 1500 diameters.

1 *A, B, C, D.* *Toxopneustes* ♀ × *Hipponoë* ♂. Normal sea water. Three longitudinal sections of anaphase of first division. Vs shown in *B* were lying beneath Vs in *A*. Chromosomes: upper plate, 34; lower, 34.

2 *A, B, C.* *Toxopneustes* ♀ × *Hipponoë* ♂. Normal sea water. Three longitudinal sections of anaphase of first division; chromosomes: 30 and 31.

3 *A, B, C.* Same; chromosomes: 34 and 32.

4 *A, B.* Same; three sections combined in two; chromosomes: 39 and 31.

5 *A, B, C.* Same; chromosomes: 33 and 33.

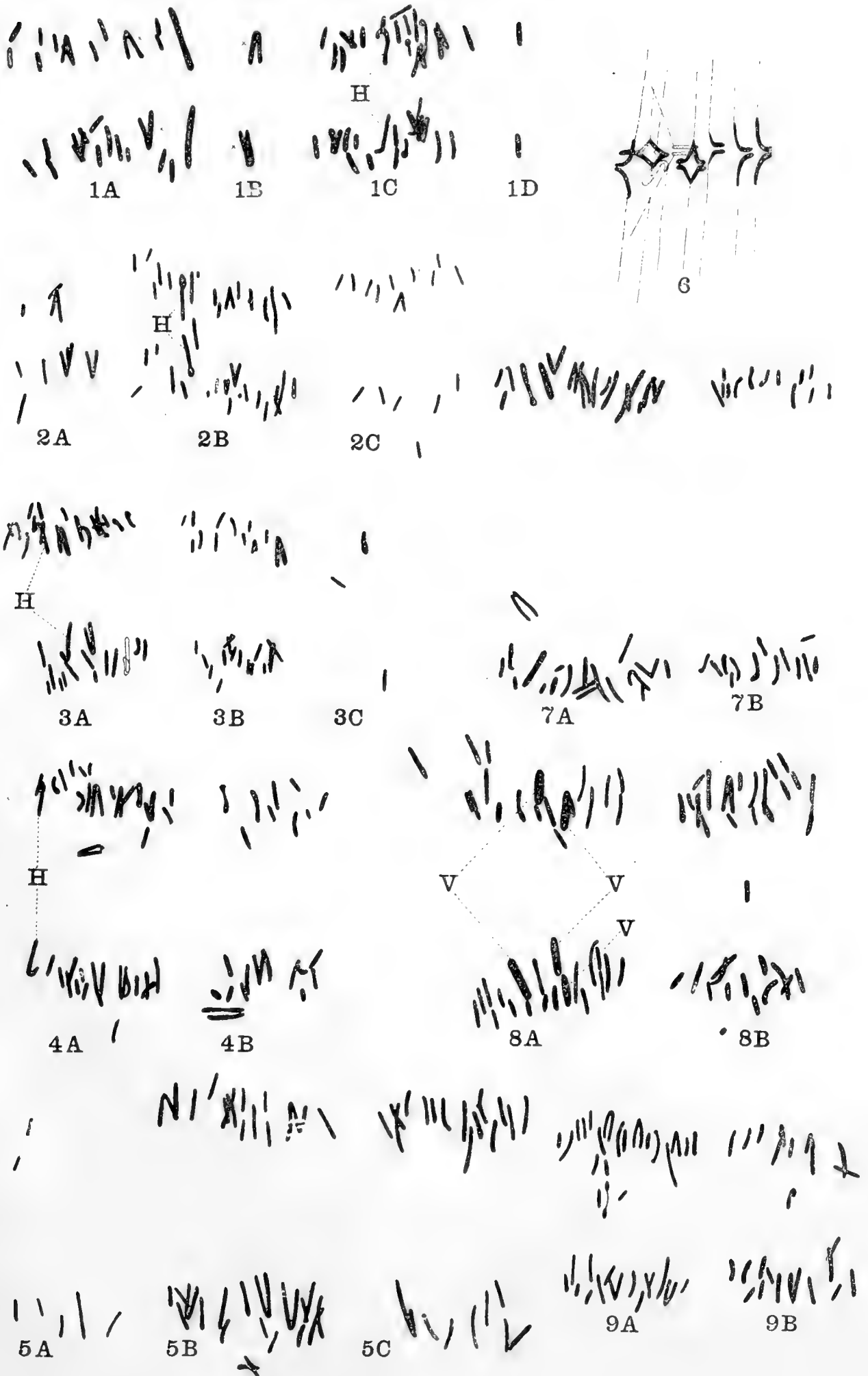
6 Same; early anaphase; part of one section.

7 *A, B.* Same; three sections combined in two; chromosomes: 33 and 34.

8 *A, B.* Same; three sections combined in two; chromosomes: 38 (?) and 35 (?). Some of chromosomes probably sectioned.

9 *A, B.* Same; sea water and acetic acid; chromosomes 28 and 29; those at center not counted.

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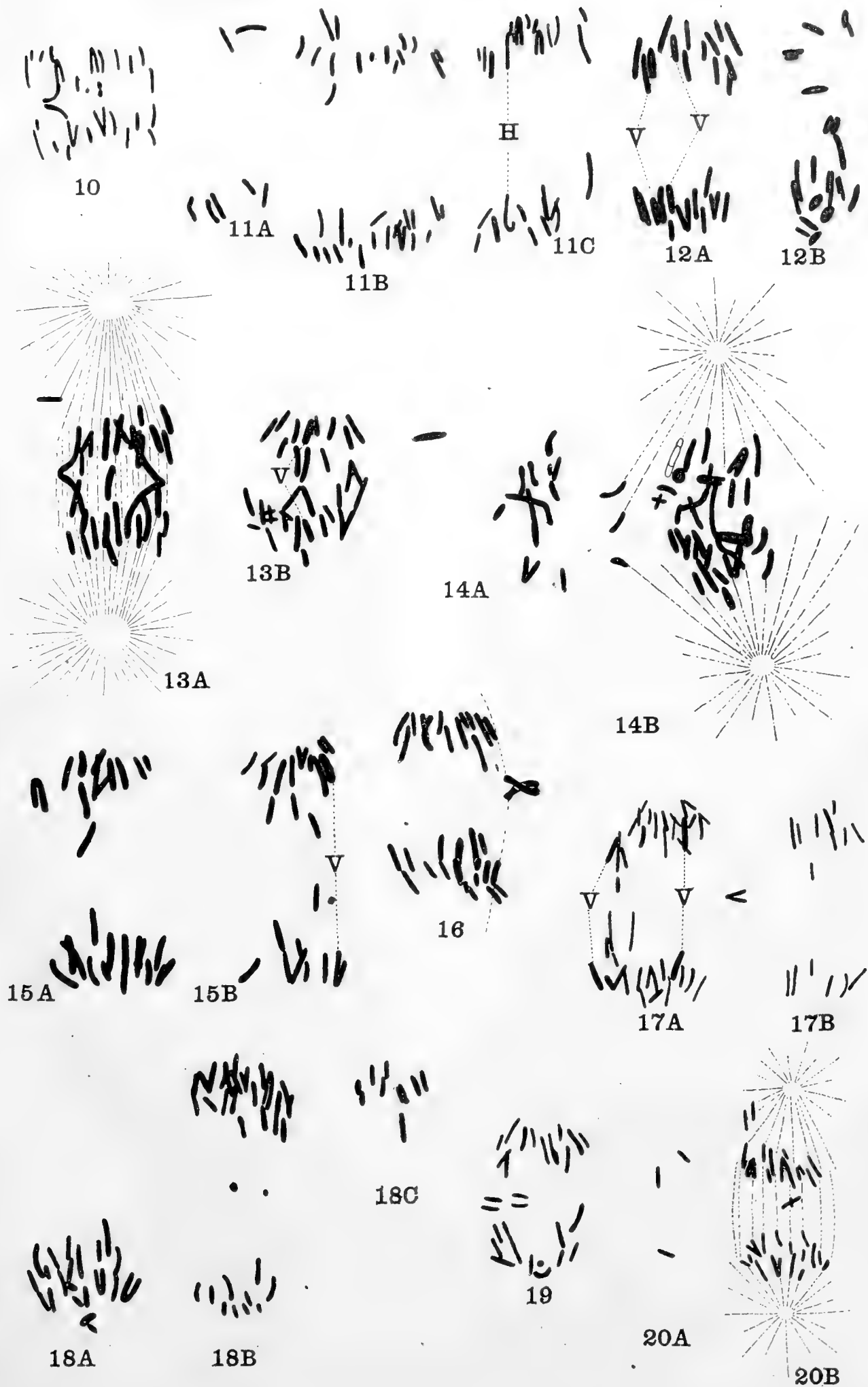


CH. H. TENNENT

PLATE 2

EXPLANATION OF FIGURES

- 10 Same; sea water and acetic acid; one section of spindle showing lagging.
- 11 *A, B, C.* Same; sea water and acetic acid; chromosomes: 30 and 35.
- 12 *A, B.* *Hipponoë* ♀ × *Toxopneustes* ♂. Longitudinal sections of anaphase of first division; chromosomes: 22 and 29.
- 13 Same; chromosomes: 30 and 36.
- 14 Same; chromosomes: 28 and 24.
- 15 *A, B.* Same; chromosomes: 27 and 24.
- 16 Same; single section showing lagging.
- 17 *A, B.* Same; anaphase of third division; chromosomes: 30 and 25.
- 18 *A, B.* Same; chromosomes: 31 and 32; those at center not counted.
- 19 Same; single section showing lagging.
- 20 *A, B.* Same; anaphase in one cell of sixteen cell stage; chromosomes: 16 and 16; two at center not counted.



D.H.T. 28

THE SKULL STRUCTURE OF DIPLOCAULUS MAGNICORNIS COPE AND THE AMPHIBIAN ORDER DIPLOCAULIA

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SEVEN FIGURES

The Permian vertebrate known as *Diplocaulus magnicornis* Cope is one of the most aberrant and specialized of all the extinct Amphibia. The species was first described by Cope from fragments of several crania and portions of the vertebral column; material which had been collected in the Permian of Texas prior to 1882. The genus had, however, been established previously on fragmentary material which had been discovered by Dr. J. C. Winslow and Mr. W. F. E. Gurley in the Pennsylvanian of Vermilion County, Illinois. The genus *Diplocaulus* was first located by Cope in 1881 among the Pelycosauria, but later researches inclined him to place the form among the stegocephalous Amphibia with relationships to the Microsauria.

The skull of *Diplocaulus magnicornis* is very peculiar in the elongation of the posterior elements of the upper surface of the cranium. The anterior elements do not take part in the posterior prolongations which give the skull such a bizarre appearance. This specialization is due to the extreme elongation of the supratemporal, the squamosal, the parietal, the epiotic and the supraoccipital.

The pineal foramen is apparently absent. I was unable to discover it on a well preserved skull in the collection of the University of Chicago (No. 2, U. of C. Collections). Cope figured it on the skull of this species which he studied in 1895. Broili says nothing of the presence of the opening and does not figure it in the restoration of the skull which he gave in 1902. Williston

was unable to locate it on the skull of *Diplocaulus limbatus* Cope which he figured in 1909.

The nostrils are located far forward on the anterior edge of the skull which curves slightly downwards so that they look forward. The orbits are small, almost circular, and are situated far forward as is the case in many of the carboniferous *Microsauria*. Other than these there are no openings on the dorsum of the skull.

The arrangement of the cranial elements is found to be approximately as Cope gave them in 1895 although there were discovered in the complete specimen evidences of the postorbital which had not been previously detected. Williston was unable to locate this element in the excellent skull of *Diplocaulus limbatus* Cope and concluded that the element behind the orbit is the postorbitofrontal (Trans. Kans. Acad. Science, 1908, pl. 1*a*) an interpretation which is open to much question. We have yet to have a definite proof of the union of these two elements, the postorbital and postfrontal, in any vertebrate skull. If there be only one present it is either one element or the other and not both. Further discussion of this will be postponed for a paper on the development of the alligator skull. The outlines of the jugal and quadratojugal were also determined. The suture separating the parietal and the supraoccipital was not found to be so erratic as Cope figured it but it continues directly across the skull. This may easily have been an individual variation. The suture separating the frontal into two equal parts was not detected although careful search was made for it. This seems to be the only case on record among the *Stegocephala* in which there has been an actual fusion of two paired elements of the cranium. Maggi has made some interesting suggestions as to the origin of the interparietal of mammals and its correlation with the fused epiotics of the *Stegocephala*. In discussing Maggi's paper it was stated that there was no case of a fusion of any of the cranial elements of the *Stegocephala* known. This I believe to be an error as is demonstrated in the present skull. This would not, however, change the decision in regard to Maggi's conclusions.

On the dorsum of the skull there were detected in several places evidences of the lateral line canals which occur as shallow grooves.

These are almost universally known as the 'slime canals of the Stegocephala,' a decided misnomer since the lateral line canals have nothing to do with the production of slime. The canals were also detected on the mandible which is associated with the present skull. On the right of the skull (fig. 1) there will be seen a distinct groove which runs along the edge of the skull. This canal had been called by Allis in *Amia* "the anterior portion of the infraorbital." There were also detected portions of the supraorbital canals but the skull is so badly crushed that it is impossible to follow the complete course of the canals.

The palate of the skull, as here given, is an advance over anything heretofore known. There still remains much to be determined in regard to its structure, but the following is offered as a contribution to the more complete knowledge of the subject. Cope, in 1895, gave a figure of the anterior portion of the palate and Broili, in 1902, gave further notes on its structure. Broili, however, had but a small portion of the skull of the animal on which to base his conclusions. Unless there be an extreme variation in the shape assumed by the skull of this species Broili's restoration is at fault in regard to the posterior curve of the skull, there being no indication of such a condition in the present specimen, nor in the species *D. limbatus* Cope. Cope gave very accurately the positions of the teeth on the palate but was unable to determine the elements which bore the teeth. In the present skull the tooth-bearing elements are found to be the premaxillae, the maxillae, the vomers, the palatines and the transverse bones.

There are five pairs of openings and depressions on the palate of the skull. These are: the internal nares, correctly represented by Cope and Broili; the palatine vacuities; the depressions which Broili calls the 'Ohrenschlitzgruben' or auditory fossae; the infratemporal foramina and depressions along the posterior lateral border of the palate which appear to be partly due to a folding over of the skull elements and doubtless gave place for the attachment of the masseter and temporalis muscles. The arrangement of the palatal openings of *Diplocaulus* does not differ in any essential respect from the condition found in the larger *Stereospondylia*.

It is worthy of note that the palatal elements in *Diplocaulus* do not take part in the formation of the prolongation of the dorsum of the skull and we get an idea of the primitive condition of the skull of the *Diplocaulidae* from the circumscribed area of the palatal elements which are restricted by the quadrates to the anterior portion of the skull so that all of the elongation and expansion has taken place in the dorsum.

The palatal openings are all lateral in position. The internal nares are the most anterior. They are small, oval and transverse in position. They are bounded by the premaxillae, vomers and palatines. The palatine foramina are large, oval openings situated below the orbits on either side of the median line. Their long axis is parallel to the axis of the skull. They are bounded by the parasphenoid, transverse, vomers and palatines. The infratemporal foramina lie anterior and somewhat medial to the quadrates. The openings have a rounded triangular form and are bordered by the transverse, the maxilla, the quadratojugal, the quadrate and the pterygoids. Posterior to the quadrate there is an elongate groove which is possibly homologous with the quadrate foramen of the Pelycosaurian genus *Dimetrodon*, of *Sphenodon*, *Anaschisma* and other stereospondylous forms. Its function here seems to be for the attachment of the masseter and temporalis muscles. It certainly has the position of the quadrate foramen in other forms. Its elongation is due to the backward growth of the epiotic horns. The other opening marked *es* in fig. 6, is undoubtedly the external auditory meatus. It represents in part the otic notch or ear slit of other *Stegocephala* so well shown in *Metoposaurus*, *Mastodonsaurus*, and *Archegosaurus*. Broili has called them the 'Ohrenschlitzgruben' and he is undoubtedly right.

The mandible of *Diplocaulus magnicornis* Cope is moderately heavy, though comparatively slight when compared to the size of the skull. The sutures on the mandible have been impossible to determine, with the exception of those bounding the articular. They show the articular to have been a triangular element. The teeth of the mandible consist of about thirty-five to forty short blunt cones. The form of tooth appears to be well adapted to

crushing shell fish, as Case suggests, and *Diplocaulus* may have fed on some of the smaller Mollusca of the Permian rivers and lakes. On the lateral face of the mandible, there is a distinct groove, the operculo-mandibular canal of the lateral line system. It would be interesting matter to determine if this canal extended entirely around the mandible.

Dr. Case in 1908 published a restoration of the entire animal, as the structure seemed to him to demand. However, Case neglected the insertion of the clavicular girdle which was already known and which would seem to indicate the presence of limbs. As a matter of fact limbs are still unknown in this species although Williston has recorded the discovery of small limbs in the closely related species *D. limbatus* Cope. That limbs will ultimately be discovered in the present species can not be doubted. The habits of the animal were undoubtedly as Dr. Case has suggested for them (Pop. Sci. Monthly, December, '08).

Paleontology teaches us nothing as yet of the ancestry of this peculiar genus of amphibians nor have we any record of its descendants. It is one of those peculiar forms which stands alone. It shows, however, characters which are more nearly those of the Branchiosauria than of the Microsauria in which order it is usually placed. The characters separating the early orders of Amphibia are essentially those of the ribs and vertebrae. The structure of the skull is essentially similar in all of the groups. We are not able, from the structure and composition of the skull, to distinguish a branchiosaurian from a microsaurian. The characters of the ribs and vertebrae are, however, perfectly constant and distinctive. Of course there are certain superficial characters of the skull which hold true for all branchiosaurs and microsaurians such as the absence or presence of sculpture of the cranial elements and the absence of the lateral line grooves from the skulls of the Branchiosauria. Other characters such as the presence of external branchiae in the Branchiosauria, the lack of endochondral ossification in the long bones and absence of clawed digits would seem to be of considerable importance.

Except for superficial characters the skull of *Diplocaulus magnicornis* Cope is essentially similar to those of the Branchiosauria

and Microsauria in structure and composition. The elongation of the epiotic regions to form the wide, fan-shaped, horns, the fusion of the frontals and the absence of a parietal foramen are individual or ordinal characters the importance of which is open to debate. That the fan-shaped horns were developed for the protection of gills would seem most absurd. If the creatures had gills the horns probably served to protect them but there is no evidence whatever that these forms were branchiate. Horns of a similar character are developed in many of the Microsauria in genera which are otherwise and structurally unrelated. Just what the development of these horns may mean is a difficult problem. The solution offered by Beecher of the significance of spines and horny excrescences indicating decadence may be a good one here but we know so very little about these creatures that conclusions would be premature.

When we consider the characters broadly we perceive that they indicate a group separation of the species of *Diplocaulus* as I have already indicated (*Geol. Mag.*, May, '09, p. 220). The characters which have been discussed ally the present genus with the Branchiosauria rather than with the Microsauria. The only character of the microsaurians which the species of *Diplocaulus* possess is the sculptured nature of the elements of the clavicular girdle and cranial elements. The characters of the ribs and vertebrae are essentially those of the Branchiosauria but they differ from these in the specialization of the zygosphenes and zygantra, which have not been detected in the branchiosaurs, and in the structure of the ribs. The fact that the ribs are borne on the middle of the centrum on an elongate transverse process would be sufficient to indicate its complete separation from the Microsauria in which the ribs are universally intercentral. The presence of an epicondylar foramen in the humerus is another distinctive character of *Diplocaulus* and entirely lacking in both Branchiosauria and Microsauria. In short the characters presented by *Diplocaulus* are so confusing and contradictory that they compel us to perceive that after all a final classification is impossible. There will always be new classifications so long as there are new forms and new intellects at work upon the material. But if we

must have a classification for convenience why not have it consistent at least? If we use a character to distinguish two orders of Amphibia like the Branchiosauria and Microsauria, and the character has been accepted for nearly half a century, then why not apply the same rule to another group of amphibians, and on the structure of the vertebrae, ribs and limb bones establish the new order Diplocaulia? It seems consistent at least if nothing more.

In pursuance of this I give here the ordinal characters of this new group of Amphibia-Diplocaulia: Skull proportionately very large with epiotic angles drawn out into fan-shaped horns, the expansion being due to the supratemporal, epiotic, parietal, squamosal and supraoccipital. Frontals fused into a single plate. Lachrymal probably absent. Pineal foramen absent. Orbits small, circular and anteriorly placed. Sclerotic plates unknown. Nostrils on or near the anterior edge of cranium. Teeth borne on mandible, premaxillae, maxillae, palatines, vomers and ectopterygoids. Teeth rounded, acrodont, denticles, abundantly present and apparently fitted for crushing hard substances. Palatal region restricted to the anterior portion of the skull. It does not take part in the epiotic prolongation. The palatal aspect of the cranium interrupted by five paired openings which are: the internal nares, the palatine foramina, the infratemporal foramina, the quadrate foramina for the attachment of the masseter and temporalis muscles and the auditory slits or external auditory meatus. The occipital condyles occur under the projecting shelf of the supraoccipital plates. Basioccipital partly cartilaginous and condyles borne by exoccipitals. Lateral line grooves present on skull and mandible.

Atlas ribless and essentially urodelous in structure. The ribs bicipital and borne on large transverse processes springing from the arch and centrum. The zygosphenal articulation not so well developed as the zygapophysial one. Vertebral formula unknown. Vertebrae elongate with low spine. Notochord but partly persistent and absent in the middle portion of the centrum, persisting as a double cone in the intervertebral regions. Clavicular girdle composed of interclavicle, clavicles and coracoids (?)

the first two of which are sculptured. These are quite large and indicate the presence of limbs in species where actual limbs have not been found. Humerus with endochondral ossifications resembling the Branchiosauria. Epicondylar foramen and muscular expansions present. Carpus and tarsus unossified. Femur elongate and somewhat twisted.

There are three species of this order known. They are:

Diplocaulus salamandroides Cope, described from fragmentary material collected in the upper carboniferous beds of Salt Creek, Vermilion County, Illinois, in 1877.

Diplocaulus limbatus Cope, described from fragmentary material from the Permian of Texas. Further descriptions and figures of the skull, girdles and limb bones given by Williston in 1909.

Diplocaulus magnicornis Cope, described from a nearly complete cranium from the Permian of Texas.

Closely related forms of this group are possibly to be found in the Crossotelidae from the Permian of Oklahoma, but the group is as yet very imperfectly known.

BIBLIOGRAPHY

- BROOM, R. 1910 Comparison of the Permian reptiles of North America with those of South Africa. *Bull. Amer. Mus. Nat. Hist.*, vol. 28, p. 214.
- COPE, E. D. 1882 Third contribution to the history of the vertebrata of the Permian formation of Texas. *Proc. Amer. Phil. Soc.*, vol. 20, p. 453.
- 1881 Catalogue of vertebrata of the Permian formation of the United States. *Amer. Nat.*, vol. 15, p. 162.
- 1882 Permian vertebrata, *Amer. Nat.*, vol. 16, p. 925.
- 1888 Systematic catalogue of vertebrata from the Permian. *Trans. Amer. Phil. Soc.*, vol. 16, p. 286.
- 1896 The reptilian order Cotylosauria. *Proc. Amer. Phil. Soc.*, vol. 34, p. 455. Pl. 9.
- MILLER, S. A. 1889 *N. A. Geology and paleontology*. p. 621.
- CASE, E. C. 1900 Vertebrates from Permian bone bed, Illinois. *Journ. Geol.*, vol. 8, p. 710.
- 1908 A great Permian delta and its vertebrate life. *Pop. Sci. Monthly*, vol. 73, p. 567, figs. 12, 13.

- BROILI, F. 1902 Beiträge zur Kenntniss von *Diplocaulus* Cope. Centralblatt für Mineralogie, p. 536.
- 1904 Permische Stegocephalen und Reptilien. Paleontographica, Bd. 51, p. 8, pls. I, IV, V.
- JAEKEL, OTTO 1903 Über *Ceraterpeton*, *Diceratosaurus* und *Diplocaulus*. Neues Jahrb. Mineral., p. 126.
- MOODIE, ROY L. 1908 The dawn of quadrupeds in North America. Pop. Sci. Monthly, vol. 72, p. 565, fig. 5.
- 1908 The lateral line system in extinct amphibia. Jour. Morph., vol. 19, p. 522, figs. 9, 9a, 10.
- 1908 Carboniferous quadrupeds. Trans. Kans. Acad. Science, vol. 22, p. 243.
- 1909 The Microsauria. Geol. Mag., Dec., vol. 6, p. 220.
- WILLISTON, S. W. 1908 The skull and extremities of *Diplocaulus*. Trans. Kans. Acad. Science, vol. 22, p. 122, pls. 1-6. Describes more fully *Diplocaulus limbatus* Cope, locates *Diplocaulus* in Microsauria.
- 1910 Dissorophus. Journ. Geol., vol. 18, p. 534. Gives list of Permian amphibia of North America.

PLATE 1

EXPLANATION OF FIGURES

1 Dorsum of skull of *Diplocaulus magnicornis* Cope. Infraorbital canal at point of arrow. $\times \frac{1}{2}$.

2 Ventral surface of the mandible. The operculo-mandibular canal at the point of the arrow. $\times \frac{1}{2}$.

3 Oblique view of one ramus of the mandible to show the entire course of the operculo-mandibular canal. Nearly natural size.



PLATE 2

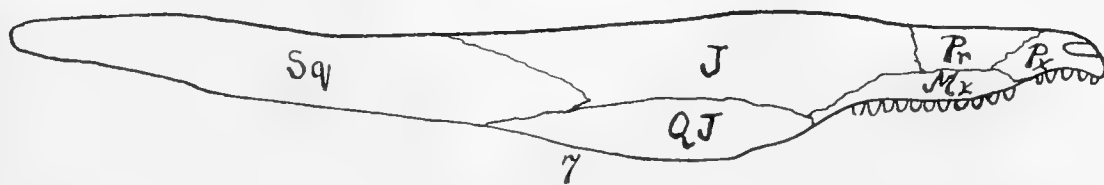
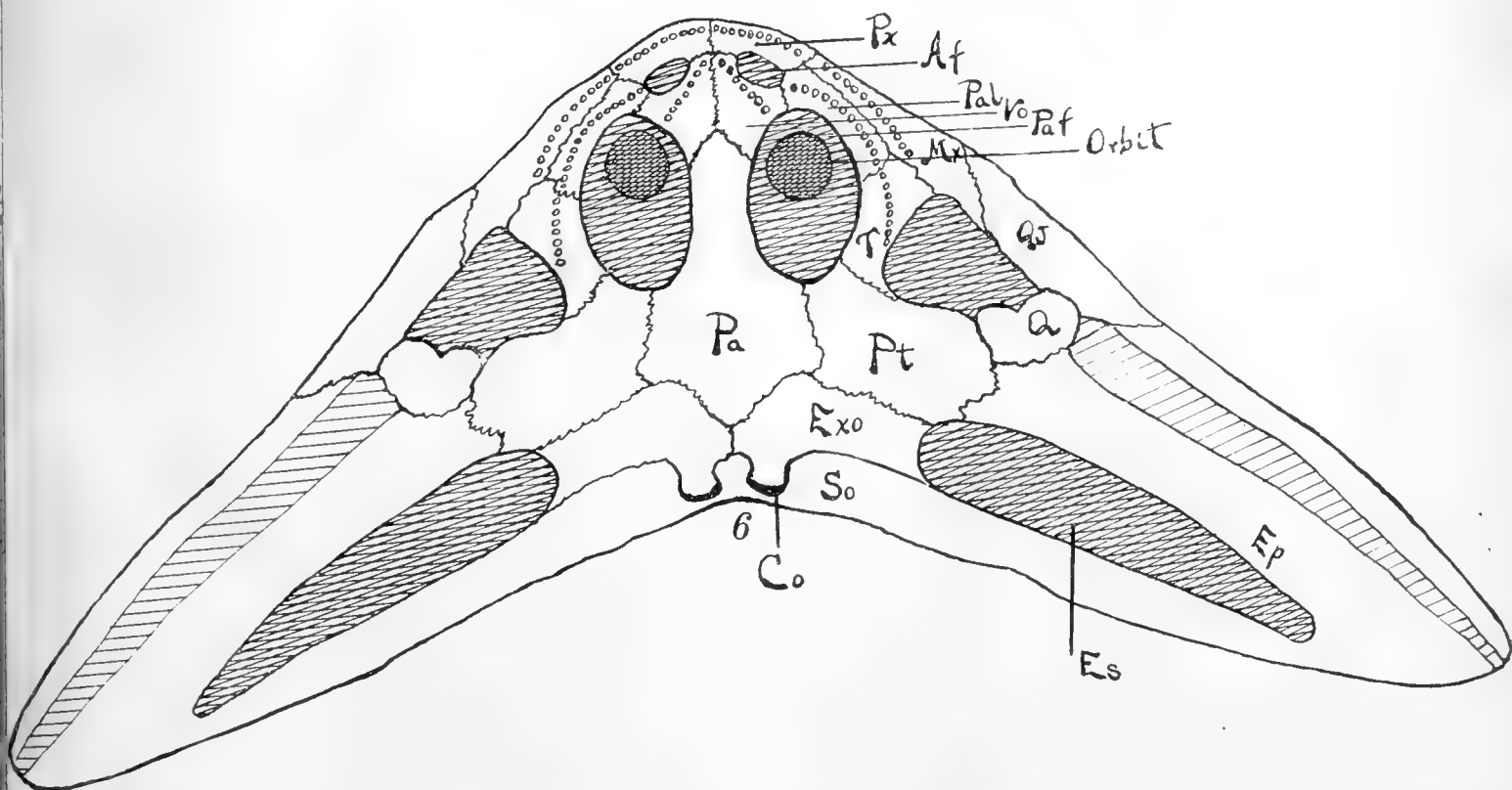
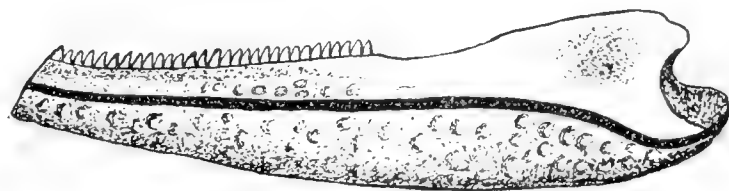
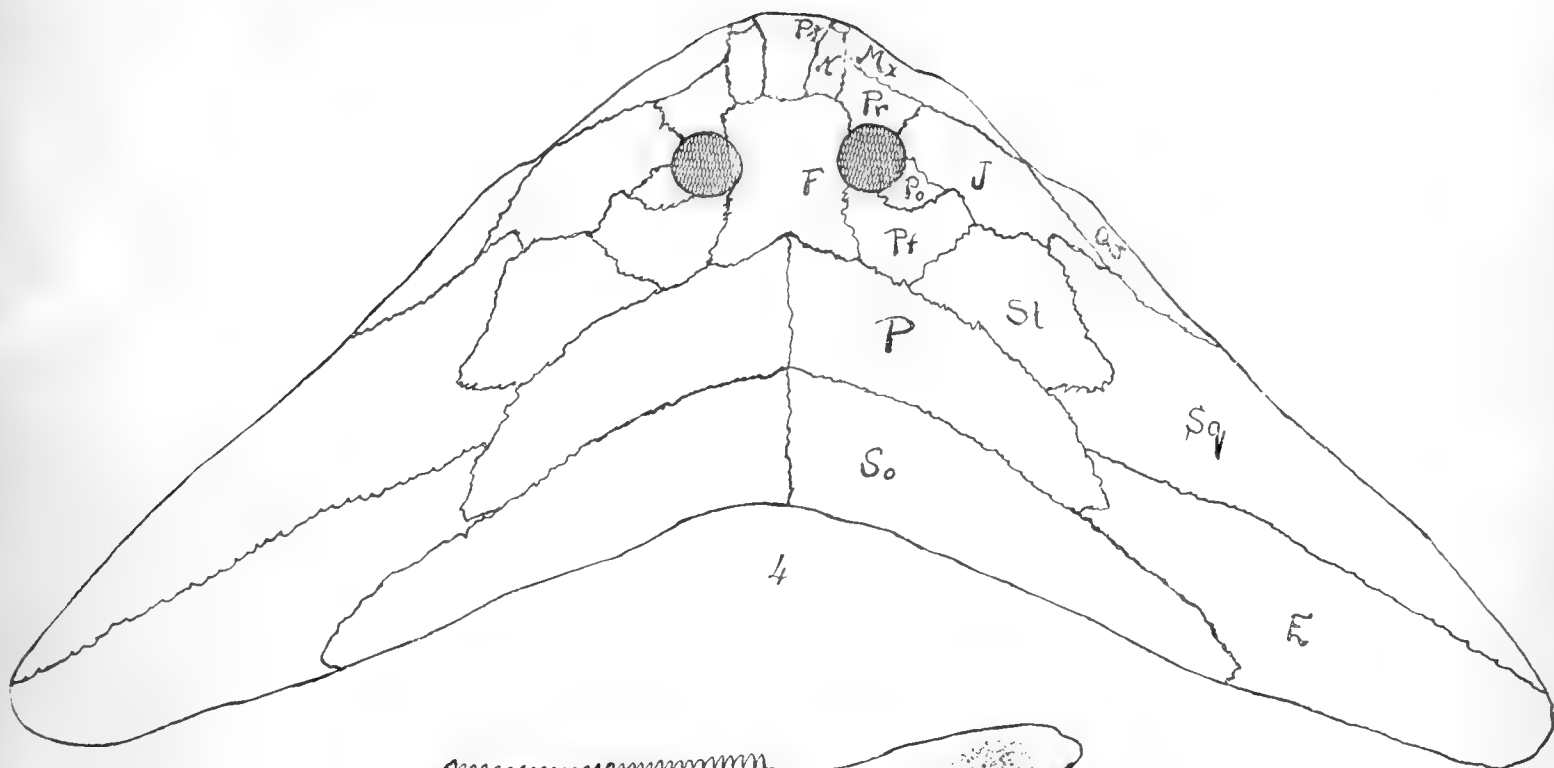
EXPLANATION OF FIGURES

4 Outline of the cranial elements of *Diplocaulus magnicornis* Cope. *E*, epiotic; *F*, frontal; *J*, jugal; *Mx*, maxilla; *N*, nasal; *P*, parietal; *Pf*, postfrontal; *Po*, post-orbital; *Pr*, prefrontal; *Px*, premaxilla; *Qj*, quadratojugal; *So*, supraoccipital; *Sq*, squamosal; *St*, supratemporal.

5 Mandible from the side to show arrangement of the operculo-mandibular canal.

6 Outline of the openings and elements of the palate of the skull. *Af*, internal nares; *Co*, condyle; *Ep*, epiotic; *Es*, auditory fossa or ear slit; *Ero*, exoccipital; *Mx*, maxilla; *Pa*, parasphenoid; *Paf*, palatine foramen; *Pal*, palatine; *Pt*, pterygoid; *Px*, premaxilla; *Q*, quadrate; *Qj*, quadratojugal; *T*, ectopterygoid or transverse bone.

7 Side view of the skull. *J*, jugal; *Mx*, maxilla; *Pr*, prefrontal; *Px*, premaxilla; *Qj*, quadratojugal; *Sq*, squamosal.



MODIFICATIONS IN THE TESTES OF HYBRIDS FROM THE GUINEA AND THE COMMON FOWL¹

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TWENTY-THREE FIGURES IN TWO PLATES

The following study is based on material obtained from four guinea-chicken hybrids, the offspring of a yearling black langshan cock and a common guinea hen three years old. There were originally five of the hybrids, all male, but during my absence from Cincinnati one died and was not preserved. An account of the general habits and appearance of these fowls together with an analysis of their peculiar color pattern has already been published.²

Three of the hybrids were killed at the age of three years, one at the age of six, and the last one died at the age of seven. When five years old the two older ones each developed a pair of sickle feathers in the tail similar to those so characteristic of the ordinary domestic cock, although previous to this time all alike possessed the simpler type of tail feathers seen in the common hen.

When young the hybrids resembled young guineas in appearance except for the fact that the legs were feathered after the manner of the langshan breed of chickens. These feathers disappeared for the most part after a few months, leaving only a few scattering ones on the legs of the adults. All of the hybrid fowls were infertile. Two of them were kept at the Cincinnati Zoological Garden for a number of years in a large inclosure with

¹ Prepared for The Whitman Memorial Volume, but received too late to be included.

² Guyer, M. F.: Atavism in guinea-chicken hybrids. Jour. Exp. Zool., vol. 7, 1909. Also, La livrée du plumage chez les hybrides de pintade et de poule. Bul. Mus. d'hist. nat., Paris, 1909.

various other gallinaceous birds, such as peafowls, pheasants, guineas, and bantam chickens. As long as both were alive they remained together constantly but after the one was killed the other attached itself to the guinea contingent of the enclosure. Whether this was due to an instinct of kinship or whether it was the result of earlier associations with the guinea mother I am unable to say.

Upon opening the body cavity the testes in three of the hybrids were found to be normal in size and external appearance. In the fourth, while the left testis was somewhat smaller than the average, the right was greatly hypertrophied, weighing 90 grams and measuring 85 mm. long by 54 mm. broad, by 30 mm. thick. The enlarged testis was kidney-shaped and lay diagonally across the body cavity. Two distinct regions, separated externally by a shallow furrow and internally by more or less of a connective tissue septum, were visible. The anterior region, representing about one-third of the testis, was a white, waxy, fatty mass. The remaining portion was somewhat more firm and was richly supplied with small blood vessels which at intervals exhibited numerous plexuses and varicosities.

Toward the anterior end of this hypertrophied organ and marked off from it as a distinct body by a constricted band of connective tissue was what appeared to be a small accessory testis. Subsequent microscopic examination showed that the left testis, the posterior region of the hypertrophied right, and this smaller accessory body all contained seminiferous tubules, although they were comparatively scarce in the hypertrophied body. Such a hypertrophied condition of one testis has been noted by other students of hybrids such as Suchetet,³ for instance, who in speaking of a hybrid duck (*C. moschata* × *A. boschas*) states that one of the two testes had the dimensions greatly exaggerated, and with its juices devoid of spermatozoa. Although, with the exception just mentioned, the testes of the hybrids appeared normal from a macroscopic examination, microscopic investigation showed them to be markedly abnormal; to such a degree in fact that no trace of spermatozoa were observable although in

³ Suchetet, André: Des hybrides à l'état sauvage; Oiseaux, Tome 1, 1896. Lille.

certain favorable regions spermatogenesis was seen to be in progress as far as the formation of spermatids. None of the latter, however, was ever found in process of transformation into the spermatozoon.

Upon examining sections of the testis the first thing to strike the attention was the great scarcity of seminiferous tubules. While a few of these were visible in sections from nearly all regions, they existed for the most part as narrow scattered tubules (fig. 1) surrounded by vast areas of intervening tissue, or more frequently as small island-like groups of a few larger tubules in a more or less homogeneous field of connective tissue and stroma cells (fig. 2). At times, however, limited areas (fig. 3) would be encountered in which the seminiferous tubules were almost as plentiful as in normal non-hybrid individuals. One hybrid in particular differed markedly from the others in having a plentiful supply of tubules throughout the most of the testis. In certain of these tubules division stages of spermatogonia and first spermatocytes were plentiful. Less frequently dividing spermatocytes of the second order were found. Side by side with such tubules, and often in greater numbers, would be found other tubules in process of degeneration.

The seminiferous tubules of the guinea have a thicker investing rind or wall than do those of the ordinary domestic cock, and while there is considerable fluctuation in the diameter of different tubules in both species, on the whole, the former has a noticeably broader, coarser looking type of tubule. There is also slightly more interstitial tissue in the testis of the guinea. In all of these respects, as well as in the general appearance of the various mitoses the hybrids approximate more nearly the condition found in the guinea.

In the hybrids, as already stated, the seminiferous tubules were usually few in number and widely separated by intervening tissue. Under the low power of the microscope this inter-tubular field ordinarily had a homogeneous cellular appearance, but under a moderate or high power the cells were seen to be arranged in irregular cords or strands, although this corded appearance often graduated indistinguishably into a homogeneous field. The stroma or interstitial cells occupying most of the inter-tubular

field are for the most part characterized by their round, plump, slightly eccentric nuclei with a comparatively large, well-marked sphere of denser appearing cytoplasm at one side (fig. 10). In places, however, they resemble closely the spermatogonia lining the inner wall of the tubules and, apart from location, are practically indistinguishable from them. Not infrequently, moreover, scattered here and there among them are to be seen typical spermatocytes in process of division. Such areas appear to be the remains of former tubules of which the walls have become entirely obliterated and the characteristic arrangement of the tubule cells lost. Such inter-tubular areas with numerous cells exhibiting various features of the maturation phenomena were especially plentiful in the hybrid already indicated as possessing a great number of tubules (fig. 4).

The germ-cells in many of the tubules were in process of degeneration. In some cases only a peripheral row of spermatogonia and Sertoli cells remained but more commonly the cells progressed to the spermatocyte type and then halted at the point of synapsis. So common was this, indeed, that the characteristic appearance of the tubules was that of a mass of cells in the contraction phase of the primary spermatocytes (fig. 9). The deeply staining chromatin strands became massed at one side of the nucleus, commonly lying in more or less of a crescent along the inner surface of the nuclear membrane. Frequently such nuclei had a vacuolar, abnormal appearance as if on the point of dissolution. Less often the nuclei of the spermatocytes had a clear watery looking center with the chromatin spread around the periphery. Not a few syncytial masses existed containing numerous degenerating nuclei, among which there were often evidences of fusion or of direct division.

As to why so many of the cells should be unable to progress beyond the beginning of the synaptic phase I can offer no further suggestion than that made in connection with a similar study⁴

⁴ Guyer, M. F.: Spermatogenesis of normal and of hybrid pigeons. Dissertation, University of Chicago, 1900. Later published as Bul. 22, University of Cincinnati, 1903. In case this paper is inaccessible to any investigator, the author will gladly supply copies as long as his stock of reprints lasts.

carried out on hybrid pigeons from 1897 to 1900; namely, that (p. 46)

in hybrids it may be supposed that in the ordinary cells of the body, the chromosomes from the paternal and the maternal species lie side by side and carry on the customary functions of the cells but when it comes to an actual fusion of chromosomes to form the bivalent type necessary for reduction, the incompatibility of the two different plasmas renders the union incomplete or prevents it entirely.

It may be of some interest in connection with the present memorial volume to record the fact that practically all of this earlier work on hybrid pigeons was done on material supplied by Professor Whitman himself. He was particularly curious about the preponderance of males among his hybrids, and was much interested in the interpretation expressed in my doctoral thesis of 1900 to the effect that the refusal of the chromosomes of hybrids to unite in the first spermatocyte indicated that synapsis normally was a fusion of maternal with paternal chromosomes, and that, granting this to be true, in fertile hybrids in the segregation of the chromosomes after synapsis we find a plausible reason for returns in the third generation to grandparental characteristics. Since this paper has had but a limited circulation it may not be amiss to restate briefly my conclusions at that time regarding this point. In a paper in 1899⁵ I had already pointed out the fact, illustrating with a diagram, that when white ring-doves and brown ring-doves are cross-mated and their offspring interbred, there is frequently a return in color in the third generation to the grandparental types. This phenomenon we now recognize at once as Mendelian but at that time Mendelism had not yet been rediscovered. In my thesis⁴ of the next year I restated these facts of my 1899 paper a little more explicitly as follows (p. 35-36):

Offspring of the common ring dove when crossed with the white ring dove are brown in color. One member of the resulting pair is frequently a few shades lighter in color than the other. In the next or third generation there is generally a return to the original colors of the grandparents; one of the young is white, the other brown. Occasionally both of the young are brown or, less frequently, both white. There is a marked tendency for the white ones to be female and the brown ones male.

⁵ Zoological Bulletin, vol. 2, no. 5; 1899.

⁴ Loc. cit.

So far as the writer has carried his experiments, the indications are that on the whole there are more brown than white birds in the third generation, and this points to the conclusion that in the brown birds we may have both intermediate forms like the hybrids of the second generation and forms which have reverted to the brown grandparent, as the white doves have seemingly returned to the white grandparent. The birds of this generation, then, might mate in such a way that the offspring could exhibit the ancestral white while yet remaining intermediate in other characters. As we shall see in the conclusions from the study of the germ-cells of hybrids, there are certain phenomena in the germ-cells which apparently afford us a definite physical basis for the production of intermediate forms and for returns to pure ancestral species. From this basis there must necessarily be a greater number of intermediate forms in the offspring of hybrids than there are reversions to the respective ancestral species.

I had interpreted the irregular phenomena occurring in hybrids at the time of synapsis as due to the tendency of the chromatin of each parent to retain its own individuality. And while I had attributed some importance to these irregularities of division in accounting for variations and reversions in the third generation, I did not regard them as the chief factors in such returns, as is evident from the following excerpt (p. 47):

In discussing irregular divisions, however, it must not be forgotten that many apparently normal divisions of the spermatocytes also occur in hybrids, and constitute by far the predominant kind of division in hybrids from closely related forms. Unequal distribution of chromatin can not therefore play the most important part in variation or reversion. There seems to be no other interpretation, indeed, than that in the many *normal* mitoses of the bivalent chromosomes which occur, the chromatin of the father and of the mother is set apart so that the ultimate germ-cells are what might be termed 'pure' cells; that is a given egg or sperm-cell contains exclusively or at least predominantly qualities from one parent. The offspring from fertile hybrids of the same parentage might then be similar to the mixed type of the original hybrid, or revert to one of the grandparent types, dependent upon the chances of the various cells for union at fertilization. If a spermatozoon and an egg containing characteristics of the same species unite, then the reversion will be to that species; if a sperm-cell containing the characteristics of one species happens to unite with an ovum containing characteristics of the other species, then the offspring will be of the mixed type again. By the law of probability the latter will be the more prevalent occurrence, because there are four combinations possible, and two of the four would result in the production of mixed offspring, while only one combination could result in a return to one of the ancestral species.

This, it will be seen, is a close approximation to the Mendelian statement of germinal segregation and combination. The qualifications necessary to bring it more strictly within the pale of Mendelism as then known were made in a brief paper⁶ early in 1903.

As previously stated, in many of the seminiferous tubules of the guinea-chicken hybrid synapsis occurred and occasionally some cells progressed to the completion of the second division and the formation of spermatids. But even where synapsis was effected there was more or less of a tendency for the union to be incomplete or partial. This was evidenced by the unusual bipartite appearance of the conjugated chromosomes and in an occasional excess of chromosomes over the number (nine) characteristic of the corresponding stage in the chicken or the guinea. In cases of such excess the extra chromosomes had the smaller size of the univalent type.

Even in normal spermatogenesis one not infrequently encounters fluctuations in the number of chromosomes. This is true to such an extent indeed that, judging from my own studies on the chromosomes of various birds and man, one is led strongly to the opinion that in these instances at least we are dealing with compound chromosomes which may occasionally resolve themselves into simpler components and in consequence exist in greater than the typical number when ready for division. On the other hand, instead of an increase there may be a reduction below the recognized haploid number, as I have shown to be true in man where the spermatocytes of the second order typically appear as five (or seven with the accessories) instead of the expected ten (or twelve); or in the case of the guinea, the chicken, and the pigeon, four (five with the accessory) instead of eight.

While in the guinea-chicken hybrids the secondary spermatocytes tend to exhibit four (or five) chromosomes in division, there is more irregularity than in the normal fowl. If my interpretation that the fusion between guinea and chicken chromosomes in the primary spermatocytes is inhibited in some way because of their inherent dissimilarities be correct, the same fact might account likewise for the occasional increase of numbers in the

⁶ The germ-cell and the results of Mendel. Cincinnati Lancet-Clinic, May 9, 1903.

second spermatocytes, inasmuch as they also would likely each contain chromosomes of different parentage.

By far the greatest number of division stages to be seen in the hybrid testis were those of the first spermatocytes. These were comparatively plentiful and to my surprise were much more normal in appearance than corresponding division stages in pigeon hybrids from more closely related species. The multipolar spindles so characteristic of the first spermatocytes in hybrid pigeons were very seldom encountered in the guinea-chicken fowls.

Perhaps the most interesting feature of this first meiotic division was the appearance of the accessory chromosome or X-element. This chromosome, whenever favorably located for observation, was found invariably to be of the guinea and not of the chicken type.

It will be recalled that in various species of invertebrates the X-element of the male is now known to be represented in the female by two such elements in all cells with the diploid number of chromosomes. In such females, after the reduction divisions each egg thus comes to have a single X-element whereas, since there was only one such element in the somatic and early germ-cells of the male, and inasmuch as this body does not divide in one of the maturation divisions of the cell but goes entire to one of the daughter cells, the X-element is lacking in half of the spermatozoa.

In all known cases where an X-element exists in the male, the eggs fertilized by a spermatozoon *without* the X-element are the ones that give rise to the new males, hence the subsequent X-element of one of these new individuals can only be one which was originally in the egg. The fact that the male zygote must always receive the X-element from the mother was pointed out by Wilson⁷ in 1906. In the present instance, since it was the mother of the hybrid that was the guinea, the X-element of the hybrid should be of the guinea type, and such, in fact, was found to be the case.

The chicken and the guinea types of X-element are readily distinguishable, that of the chicken being typically of larger

⁷ Wilson, E. B.: Studies on chromosomes, III. Jour. Exp. Zool., vi, 1906.

size, of stouter build, and of different shape. While each usually appears as a curved body, the chicken X-element (figs. 5, 11, 12, 13, 14) is more curved than the other, having a U-shape with both ends of the loop of the same size, while the guinea X-element (figs. 6, 15, 16, 17, 18) is more comma- or pistol-shaped with one end noticeably narrower than the other. While there may be greater or less deviation from these types, generally in the nature of unusual elongation or compression, on the whole, after the observer's eye has become accustomed to the elements in question, he has little difficulty in readily identifying the two types. The X-element of the hybrid (figs. 7, 19, 20, 21, 22, 23) is clearly of the guinea type. In both the chicken and the guinea the X-element, instead of having its more characteristic appearance, may occur occasionally as two closely apposed spherical chromosomes. Under such circumstances the two components are of approximately the same size in the chicken, whereas one is always noticeably smaller than the other in the guinea. The same modification may obtain in the hybrid (fig. 22), but here again the double element is of the guinea type.

While it is very difficult to secure representative appearances of the X-element of these fowl by photography because of differences in the focal plane of its different parts, still the pictures obtained⁸ are sufficiently clear to demonstrate the point in question. Fig. 5 (5*a* magnified 750, and 5*b*, 1500 diameters) is a photograph of the X-element of the langshan cock. In the photograph the typical U-shaped element appears to be more of a V, but this is due to the fact that when the extremities of the chromosome were in focus as they are in the picture, the bend of the U was below the plane of focus and thus made to appear sharp-angled. Fig. 6 shows in the guinea the metaphase of a dividing first spermatocyte viewed from one pole. The X-element at the top of the field, is plainly seen to be narrower at one end. The focus was such that its curved condition is not visible in the photograph. Fig. 7 shows a dividing first spermatocyte of the hybrid, viewed from one pole. What appears to be a long curved body at the

⁸ The writer makes grateful acknowledgment to Dr. Charles Goosmann for the microphotographs of Plate I.

top consists really of two chromosomes; one, at the base, a deeply-staining, rounded, ordinary chromosome, and the other the curved X-element. In order to show the curve of the latter the camera had to be so focussed as to blend the two images. The X-element of the hybrid, like that of the guinea, is seen to be narrower at one end.

In my paper on the spermatogenesis of the chicken,⁹ I noted the fact that it was not uncommon to find what appeared to be a tripartite accessory, but I am now inclined to believe that when such a body exists it is the X-element plus one member of a characteristic pair of small chromosomes, the other member of which passes to the opposite pole, either slightly in advance or at the time of the regular division of the ordinary chromosomes. Figs. 7, 9, 11 and 12 of that paper⁹ show evidence of this condition. In fig. 11 the small element is completely detached. Fig. 10 probably represents a condition in which the opposite member of this small pair stands apart from the equatorial plate towards one pole, the accessory being seen on the other side of the equatorial plate. In the guinea the corresponding pair of small chromosomes (see figs. 11 and 12 of my paper¹⁰ on the spermatogenesis of the guinea) is considerably smaller than in the chicken. The one which passes to the pole also reached by the X-element shows less tendency to unite with the latter than in the chicken, hence it is only rarely that a tripartite condition of these bodies is observed in the guinea.

In a paper¹¹ written in 1909 before I had discovered the presence of an X-element in the common fowl, I suggested that if we assume, as some investigators have done, that increased nutrition favors the production of females, diminished nutrition, the production of males, then the excess of males among hybrid birds might be due to the fact that in hybrids, "there would in all probability be more or less default in the metabolic processes because of the incompatibilities which must necessarily exist between two

⁹ Guyer, M. F.: The spermatogenesis of the domestic chicken (*Gallus gallus* dom.). *Anat. Anz.*, xxxiv, 22-24, 1909.

¹⁰ Guyer, M. F.: The spermatogenesis of the domestic guinea (*Numida meleagris* dom.). *Anat. Anz.*, xxxiv, 20-21, 1909.

¹¹ Guyer, M. F.: On the sex of hybrid birds. *Biol. Bul.*, xvi, 4; 1909.

germ-plasms so dissimilar." The discovery of an X-element, however, together with the knowledge that it is of maternal instead of paternal origin possibly gives us a simpler and more plausible explanation. It is the spermatozoon *without* the large X-element which unites with the egg in the production of the new male, and since such a spermatozoon is much smaller than one of the other type, the whole question may resolve itself into a mere matter of the relative sizes of the spermatozoa. For inasmuch as such hybrids are obtained with difficulty even under the most favorable conditions we may reasonably suppose that the egg-plasm is more or less resistant or antagonistic to the entrance of a foreign sperm, and that because of this the smaller type of spermatozoon enters more readily, with the result that a male is produced.

SUMMARY

1. With one exception, where one testis was greatly hypertrophied, the testes of the four guinea-chicken hybrids examined were of normal size.

2. Microscopic examination showed them to be abnormal, however. No spermatozoa were developed and the seminiferous tubules were few in number in most regions of the testis and often contained disintegrating and defective cells.

3. As in hybrid pigeons the critical point seemed to be the synaptic phase, the chromosomes of different parentage seemingly being unable to unite normally in many instances.

4. In spite of this difficulty, however, not a few first spermatocytes succeeded in passing through synapsis and subsequent division with more or less of an appearance of normality.

5. An accessory chromosome or X-element of the guinea (maternal species) type is present.

6. The X-element is of large size in the common fowl and consequently the mature spermatozoa without it are much smaller than the ones which bear it. It is suggested that inasmuch as males are produced only from eggs fertilized by a spermatozoon without the X-element, the great preponderance of males among such hybrid offspring may be due to the simple fact that the smaller type of spermatozoon can more readily penetrate a foreign, and hence more or less incompatible, egg-plasm.

PLATE 1

EXPLANATION OF FIGURES

1 Section of a testis of a guinea-chicken hybrid showing the narrow, atrophied seminiferous tubules separated by wide areas of interstitial substance. $\times 75$.

2 Section of a testis of a guinea-chicken hybrid showing an island-like mass of seminiferous tubules, normal in size, in a broad field of connective tissue and stroma cells. $\times 75$.

3 Section showing an area of the testis in one of the guinea-chicken hybrids in which, in places, the seminiferous tubules were almost as plentiful as in normal non-hybrid individuals. $\times 75$.

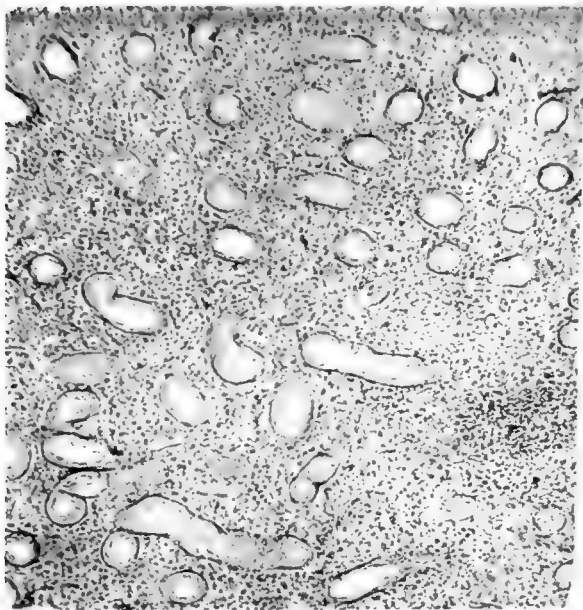
4 Section of a testis of a guinea-chicken hybrid showing an inter-tubular area in which numerous cells were in various stages of maturation. Primary spermatocytes in process of division were not uncommon. $\times 75$.

5 Metaphase of a dividing first spermatocyte of the langshan cock, fig. 5a being at a magnification of 750, and 5b, 1500 diameters. The typical U-shaped element appears to be more of a V in the photograph, but this is due to the fact that when the ends of the chromosome were in focus as they are in the photograph, the bend of the U was depressed below the plane of focus, giving to the picture a sharp-angled appearance which the real object did not possess.

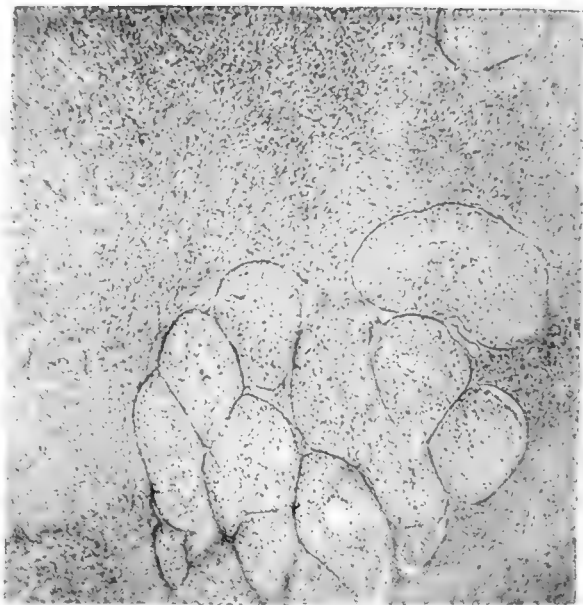
6 Metaphase of a dividing first spermatocyte of the guinea, viewed from one pole. The X-element is at the top and is plainly seen to be narrower at one end than at the other. It lay in such a position that its curved condition could not be shown by photography. $\times 1500$.

7 Metaphase of a dividing first spermatocyte of the hybrid, viewed from one pole. What appears to be a long curved body at the top consists really of two chromosomes; one, at the base, a deeply-staining, rounded, ordinary chromosome, and the other the curved X-element. To show the curve of the latter the camera had to be so focussed as to blend the two images. Like that of the guinea, the X-element of the hybrid is seen to be narrower at one end. $\times 1500$.

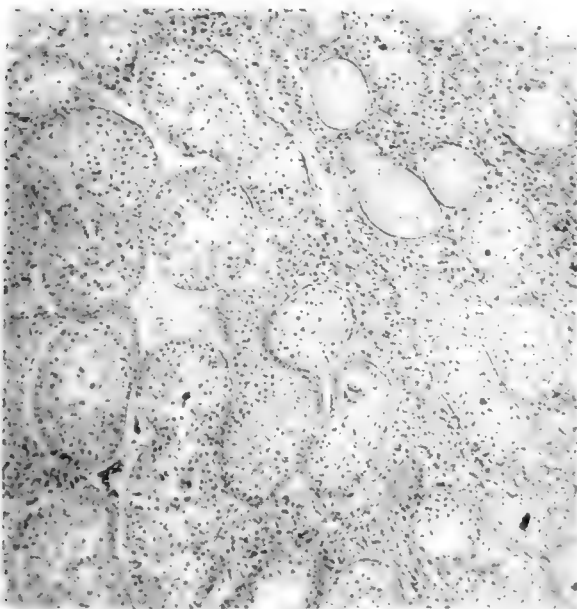
8 Side view of a first spermatocyte in the hybrid showing the cell ready for division with the X-element lying just above the level of the regular equatorial plate of chromosomes. The photograph does not reveal the curved shape of the element although this could readily be detected by manipulation of the fine adjustment of the microscope. $\times 1500$.



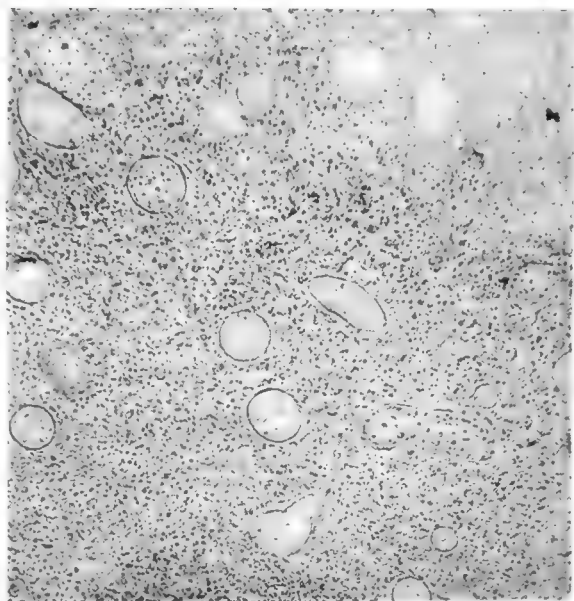
1



2



3



4



5 a



5 b



6



7



8

PLATE 2

EXPLANATION OF FIGURES

All of the drawings in this plate were made with the aid of a camera lucida although in most cases, in order to show anything of the curved nature of the X-elements or the full field of chromosomes, the focal plane had to be shifted, so that most of the drawings from fig. 11 to fig. 22 represent composites of two planes of focus. The magnification is in every case approximately 1800 diameters.

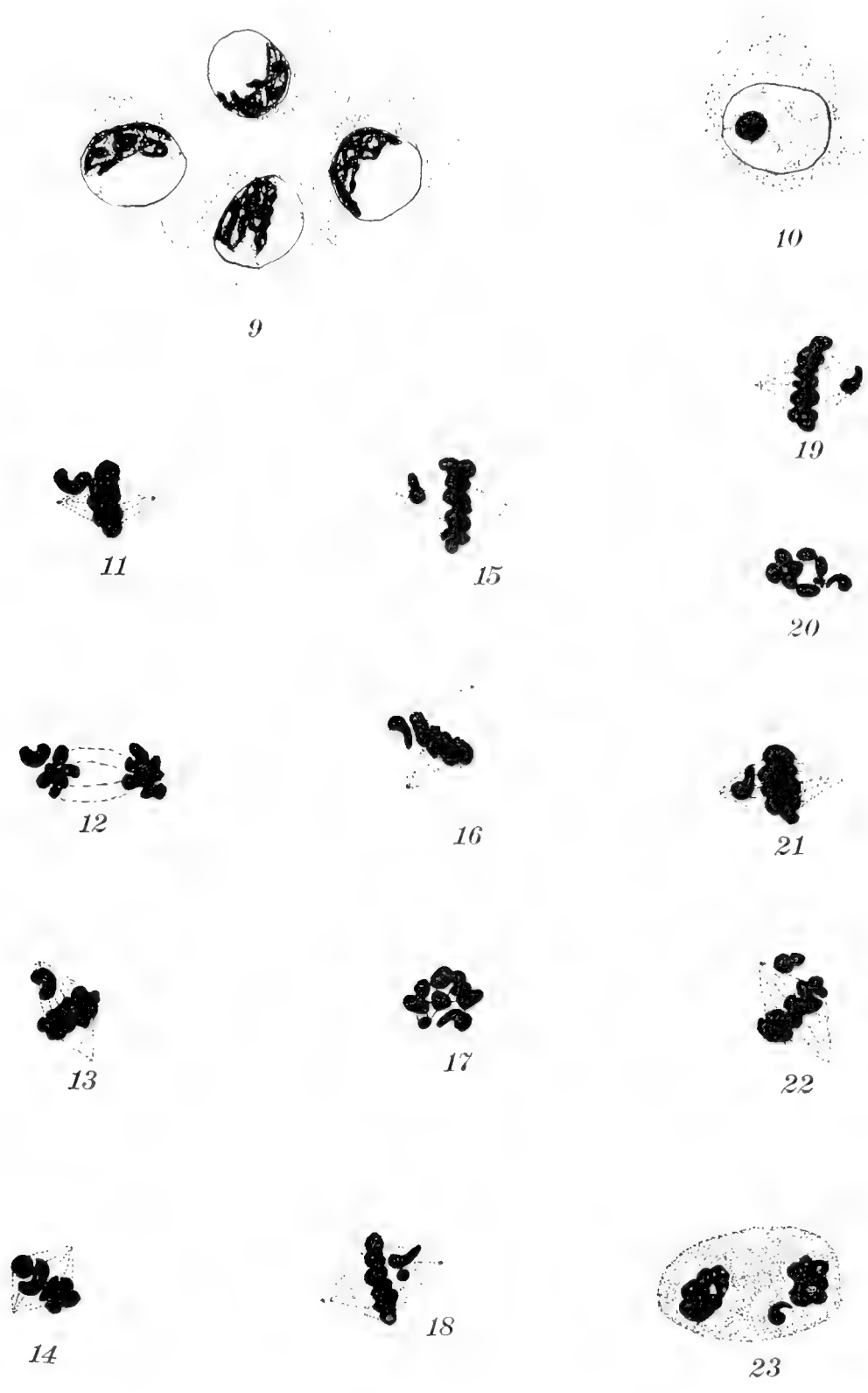
9 First spermatocytes of one of the hybrids showing the prevalent contraction phase at which the maturation process commonly came to a halt.

10 A stroma cell from the testis of one of the hybrids.

11, 12, 13, 14 Drawings showing the characteristic U-shape of the X-element of the langshan cock. Figs. 11, 12 and 13 represent division stages of first spermatocytes. Fig. 14 is from a smear preparation and shows the X-element at the equator of the spindle in a secondary spermatocyte ready for division. It divides lengthwise at this time.

15, 16, 17, 18 Drawings showing the characteristic comma- or pistol-shaped X-element of the guinea. Fig. 17 is viewed from one pole.

19, 20, 21, 22, 23 Drawings to show the X-element of the guinea-chicken hybrid. it will be observed that the X-element is of the guinea type. Fig. 20 is viewed from one pole.



THE EMBRYOLOGY OF CRYPTOBRANCHUS ALLE- GHENIENSIS, INCLUDING COMPARISONS WITH SOME OTHER VERTEBRATES

I. INTRODUCTION; THE HISTORY OF THE EGG BEFORE CLEAVAGE

BERTRAM G. SMITH

From the Zoological Laboratory of Columbia University

FIFTY-SIX FIGURES

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I. INTRODUCTION

For more than a generation zoologists have eagerly sought for the embryological material of the hellbender, *Cryptobranchus allegheniensis* Daudin. Until quite recently these efforts have been conspicuously lacking in success. It seems remarkable that the life history of an animal so large, so abundant in localities easy of access, and so important from a phylogenetic point of view, should so long remain shrouded in mystery. But the same difficulty has been encountered in attempts to work out the natural history of several nearly related forms. Eycleshymer ('06) says:

After years of persistent and patient effort Professor Whitman finally discovered the nests and eggs of *Necturus*. Only those who have for years been baffled in their attempts to obtain the embryological material of other North American urodeles, such as *Siren*, *Amphiuma*, and *Cryptobranchus* can properly appreciate the enormity of the task.

In the case of *Cryptobranchus* the difficulty in finding embryological material seems to have been enhanced by the unusual breeding season of the animal; the eggs are laid in the fall, while most amphibia spawn in the spring. Townsend ('82) published a general description of some fertilized eggs which he states were deposited in August. McGregor ('96) described very briefly an embryo 16 mm. in length, and ('99) stated that the eggs are deposited in August and September. Yet the information thus acquired in regard to the time of spawning seems not to have become generally known to others who were searching for the eggs. A suggestion might have been obtained from Sasaki's ('87) observation that the Japanese 'giant salamander,' *Cryptobranchus japonicus* (*Megalobatrachus maximus* Schlegel), deposits its eggs in August; but this also seems to have been overlooked. Reese ('04) succeeded in obtaining some unfertilized eggs, of which he gave the first detailed description.

The embryological record for *Cryptobranchus allegheniensis* remained almost a blank until 1906, when I published a preliminary report containing, besides a description of the sexual elements, a brief account of the external development during the cleavage stages. A later contribution (Smith, '07), devoted chiefly to the habits, more particularly the breeding habits, included a very general account of the life history.

From a phylogenetic point of view great interest attaches to the amphibia; there is no doubt that they lie close to the extinct ancestral stock of the highest forms of vertebrate life. Concerning the origin of the amphibia themselves Kingsley ('99) says: "All the facts of structure and development go to show that the amphibia have arisen from the crossopterygian ganoids, and that existing groups have descended from the stegocephali, each by its own line of ancestry." But when we inquire further, and attempt to trace more particularly the origin of any group of existing amphibia from an extinct form exhibiting affinities to the crossopterygii, we are landed at once in the midst of uncertainties. Confining our attention to the urodeles, we are confronted with the difficult question of the phylogenetic relationships of the different members of this group. The problem will be more

fully discussed in a later section; for the present it will be sufficient to call attention to one of its leading aspects. From existing urodeles we may select a series of forms illustrating all stages in a transition from an aquatic to a terrestrial mode of life, or vice versa. In which direction should the series be read? Or have we stated the question incorrectly, and have the urodeles reached their present condition, some from an aquatic, some from a terrestrial ancestry?

In studying this aspect of the phylogenetic problem our attention cannot fail to be attracted by *Cryptobranchus*. For here we have a urodele whose entire life is spent in the water, characterized by persistent gill slits, the most primitive brain (Osborn, '88), and external fertilization (Smith, '07). On the other hand *Cryptobranchus* is known to possess deciduous external gills, functional lungs, and a method of locomotion by crawling on the bottom which suggests a former terrestrial habit. Is *Cryptobranchus* primitively aquatic, or does it come down to us bearing evidence of a former land-living existence? An answer to this question would go far in advancing our knowledge of the phylogeny of the entire group.

In the solution of our phylogenetic problem comparative anatomy, paleontology and embryology must work together. It is the embryological evidence that has hitherto been most conspicuously lacking. Notwithstanding the important position of the aquatic urodeles, it is here that we find one of the widest gaps in our knowledge of comparative embryology. Not only has the development of *Cryptobranchus allegheniensis* remained undescribed, but little or nothing is known concerning the embryology of most of its near relatives. Very recently, it is true, considerable progress has been made in the study of the embryology of *Cryptobranchus japonicus*, but part of this work was done on very scanty material, and the field is by no means exhausted. Of the development of *Amphiuma* and *Siren* practically nothing is known. Some results have been obtained with special problems in the development of *Necturus*, but the life history has not been covered in a comprehensive manner. For a study of the phylogenetic relations of these forms a knowledge of the development in its

general aspects as well as along special lines is imperative; and in no other form do the embryological data promise to shed greater light on phylogenetic problems than in the case of *Cryptobranchus*.

For the analysis of developmental processes from a morphogenetic point of view the eggs of *Cryptobranchus* present certain favorable features. One of these is the presence of a larger amount of yolk than is known in the egg of any other amphibian; they are thus favorable objects for the study of the influence of yolk on development. The eggs, moreover, are lacking in pigment, and the early segregation of the yolk gives a translucency to parts of the embryo even in the gastrula stage, enabling one to study satisfactorily in the living egg some of the internal features of development. The embryo is found to respond admirably to the influence of chemicals modifying the course of development; for certain experiments of this sort it gives results decidedly more definite than have been obtained with the embryo of the frog.

The present contribution is to be followed by other parts dealing with the embryonic and larval development.

This investigation has been pursued under a great variety of circumstances, and with many protracted interruptions due to the pressure of other work. Field work on the habits of *Cryptobranchus*, the collection and preservation of material, and the study of the living egg, have been carried on each autumn ('05-'11 inclusive) in northwestern Pennsylvania. For comparison with *Cryptobranchus*, I have collected embryological material of *Necturus* during the seasons of 1910 and 1911, from Lake Monona, Wisconsin. Laboratory work, principally on preserved material, begun in the Zoological Laboratory of the University of Michigan ('05-'07), has been continued in the zoological laboratories of Lake Forest College ('07), Syracuse University ('08-'09), the Bureau of Fisheries at Woods Hole (summer of 1908), the University of Wisconsin ('09-'11), and Columbia University ('11-'12). To the directors of these respective laboratories I wish to express my sincere thanks for uniform courtesy in placing the resources of each institution at my disposal. To Professor Bashford Dean, under whose direction the work is being

carried on during the present year, I am profoundly indebted for his constant encouragement, kindly criticism, and valuable advice; for this I desire to record my grateful appreciation.

II. THE ADULTS

A. HABITAT

Cryptobranchus allegheniensis was found abundantly in the Brokenstraw Creek, a tributary to the Allegheny River, in north-western Pennsylvania. The most favorable locality extends from the confluence with the Allegheny five or six miles upstream. The stream has a rather rapid descent, and a gravelly or rocky bottom. Shallow and rocky rapids make up the greater part of its course, alternating with areas of deeper and more quiet water.

As a rule, *Cryptobranchus* is found more abundantly in rather shallow and rapid water, where large flat rocks afford suitable cover. Usually the animals lie concealed in cavities under these rocks. As more than one individual is seldom found under a single rock, we conclude that its life is in general a solitary one. *Cryptobranchus* rarely comes out of its hiding place in the daytime, except in the spring and early summer and during the breeding season (the first two weeks of September). At night they venture abroad in large numbers; they are then seen by fishermen spearing by torchlight, who commonly report them in the deeper and more quiet water.

The cavity or cavern used for a more or less permanent dwelling-place has a rock for its roof and the gravelly bed of the stream for its floor. In perhaps the majority of cases, ready-made cavities are chosen as homes, and these are reached by a natural opening. But the cavity sometimes bears evidence of having been in part hollowed out by the animal, and is occasionally reached by a single tunnel-like entrance on the down-stream side of the rock; this is more often the case in cavities used for spawning purposes.

There is a striking similarity between the habitat of *Cryptobranchus allegheniensis* and that of the 'giant salamander' of Japan as described and figured by Ishikawa ('04).

B. GENERAL CHARACTERISTICS

1. *Size*

Out of the many hundreds of adults captured, the largest male (September 3, '08) measured 60 cm. ($23\frac{1}{2}$ inches) long and weighed $2\frac{1}{2}$ pounds. The largest female captured (September 3, '09) weighed exactly 3 pounds. The latter animal unfortunately escaped from the aquarium in which it was confined and was not measured; probably it was no longer than the longest male, but heavier because distended with eggs. Professor McGregor reports a specimen 25 inches long, taken from the Scioto River.

The great majority of specimens captured by me were much smaller; specimens of about 30 to 50 cm. were most frequently taken. The smallest sexually mature male measured 30 cm.; the smallest mature female 35 cm.

2. *Form*

As compared with the young, the adult is more flattened dorso-ventrally—an adaptation to life in shallow crevices. The head particularly shows this flattening: it is wedge-shaped as viewed from the side, a form which enables the animal to force its soft body into very shallow crevices.

Moreover, as compared with the young, the adult is distinguished by a general looseness and wrinkling of the skin at the sides of the body, forming broad lateral horizontal folds; and by similar flaps of skin on the posterior sides of the limbs. During locomotion these folds and flaps undulate in the water, contributing to the uncouth appearance of the animal.

3. *Coloration*

Young sexually mature individuals vary little in color or color pattern. The ground color is dull brown, with conspicuous black spots and less conspicuous yellow spots scattered over the dorsal and lateral surfaces. Both kinds of spots are irregular in size and form. The coloration of young adults is practically that of

immature specimens from 16 cm. body length upwards; in these stages the spots are more conspicuous than in the larvae or the older adults. In the older, full-grown specimens the general color effect may vary in two ways: it may become either greenish-brown or decidedly reddish brown. As stated by Reese ('03) these variations in color occur in both sexes.

C. BREEDING HABITS

1. *Breeding season*

The following data indicate the beginning of the breeding season, as shown by the deposition of eggs, in northwestern Pennsylvania during a series of years:

1906.....	August 30	1909.....	August 29
1907.....	September 8	1910.....	September 1
1908.....	August 28	1911.....	September 4

The summer of 1907 was an unusually 'late season' as regards vegetation as well as the breeding season of *Cryptobranchus*. This indicates the probability that climatic conditions influence the time of spawning.

Egg-laying continues for a period of about two weeks. At the end of this time females have in a few instances been taken with the full complement of ripe eggs still in the ovary and showing signs of degeneration.

The occurrence of the breeding season of *Cryptobranchus* in the fall is in marked contrast to the habits of nearly all other urodeles. Some other urodeles which have a late breeding season are *C. japonicus*, which according to several authors (Sasaki, '87; Kerbert, '04; Ishikawa, '04; de Bussy '04 and '05) lays its eggs during the latter part of August and the early part of September; and *Amphiuma*, which according to McGregor ('99) breeds in midsummer. Among the anura, *Scaphiopus holbrookii* spawns during the summer, the time varying from June to August (Pike, '86; Hargitt, '88).

2. External sexual characteristics

The adult male may be recognized (Reese, '04) by the presence of a swollen ring about the cloaca, due to glands beneath the skin. This swelling is quite prominent during, and for a few weeks before the breeding season. I found it difficult to distinguish by external characteristics the sexes of a few specimens taken during the first week of July; during the latter part of July the males could easily be distinguished by the presence of the cloacal swelling. In a few males obtained and examined during the early part of November, the swelling was less pronounced than is usually the case during the breeding season. Females are characterized by the entire absence of the cloacal protuberance found in the male; also, the abdomen of the gravid female is slightly swollen.

3. Sex ratio and sex segregation

As a general rule, fewer females than males have been captured. The record of the sex of the great number of adults captured during the progress of the work is not complete, but the conclusion reached by later work is that the original ratio of 1:8 determined (Smith '07) during the fall of 1906 is much too large. In a series of years the proportion of females to males captured is about 1:2 or 1:3. These results are of course not conclusive as to the actual sex ratio; as will presently be explained, the sex ratio in the specimens captured varies for different times and places, and the true ratio may be disguised by the occurrence of seasonal segregation of the females from the more accessible localities.

In studying the distribution of the sexes throughout the year a distinction must be made between localities which experience has shown are chosen as breeding grounds, and other localities unsuited for breeding purposes. The breeding grounds are characterized by shallow water, a moderate current, and the presence of large flat rocks affording cover for cavities protected from the current. Elsewhere a swifter current, smaller rocks barely large enough to serve as cover, or deeper pools of quiet water, afford conditions in which *Cryptobranchus* can live, but which are not adapted for purposes of reproduction.

Studies of the sex ratio indicate a more or less perfect segregation of the sexes at certain seasons of the year. A dozen adults captured in June on the breeding grounds, by an assistant, proved to be all males. During the summer, search of the breeding grounds results in the capture of a few females and a much larger number of males; in localities unsuited for breeding one is more likely to find females, and males are seldom found in their immediate vicinity. Just before the breeding season one is more likely to find females on the breeding grounds, but the males are still considerably in excess, and there is apparently a tendency for the sexes to occur in groups: within a restricted area one may find only males, while within another area a short distance away one may find only females. At the height of the breeding season, both sexes are found on the breeding grounds in more nearly equal numbers.

For some days or weeks after the close of the breeding season the male remains in possession of the nest; females have never been found in nests containing eggs. At this time females have been found in considerable numbers in localities unsuitable for breeding, with no males in their immediate vicinity.

The general results of the studies of the sex ratio and the distribution of the sexes indicate that the males abound in localities suitable for breeding, throughout the year, and that they are less numerous elsewhere; it is positively established that the males alone are in possession of the nests after spawning takes place; and it is probable that there is a more or less perfect segregation of the females from the breeding grounds during a period extending from the close of the breeding season until the middle of the following summer.

In *Necturus*, segregation of the sexes at a certain season of the year seems to be more complete than is ever the case with *Cryptobranchus allegheniensis*. Eycleshymer ('06) says:

In the autumn they are found in pairs or small groups. From this fact and others to be recorded later it is inferred that this is the mating season. . . . During egg-laying [in the spring] the males are never found with the females, and where they remain is unknown.

4. *The eggs*

(a). *General history of the eggs and their envelopes before the time of spawning.* In an adult female of average size about 450 eggs are matured each season—225 from each ovary. In general the number is greater in the larger and presumably older females than in the smaller ones. At the approach of the breeding season the eggs which are about to become mature are readily distinguishable from the others by their much greater size and yolk content. The liberation of these eggs from the ovary and their passage down the oviduct takes place shortly before spawning. The exact date varies considerably in different individuals; for a week or ten days after the first cases of spawning, females may be found with mature eggs all still in place in the ovaries. The process of liberation of the eggs and their passage down the oviduct, once begun, must be accomplished with considerable rapidity; for out of more than a hundred females opened and examined during the breeding season in the course of several years, only four have been found in which the process was actually taking place. This state of affairs is in marked contrast to the condition in *Bufo*, where according to King ('05) the great majority of specimens collected soon after they had emerged from their hibernation contained eggs free in the body cavity. In three out of the four cases above mentioned for *Cryptobranchus*, eggs were found along the entire route: some still in place in the ovary; some free in the body cavity, for the most part collected at its anterior end, near the opening of the oviduct; others forming a procession down the oviduct; the remainder aggregated in the uterus. In the fourth case, the ripening eggs were found only in the body cavity, oviduct and uterus. The process takes place on the two sides of the body simultaneously.

During their passage down the oviduct the eggs receive their gelatinous outer envelopes, the product of the oviduct. At the upper end of the oviduct, the eggs collect in masses; a little further down, they are arranged in a solid row. In these parts of the oviduct the covering is absent or just beginning; the eggs are very soft, and elongated by pressure of the walls of the oviduct. In

the middle and lower portions of the oviduct the eggs are distributed at fairly equal intervals; here the envelope is well formed, and consists of a capsule about each egg, and a slender connecting cord, giving a general resemblance to a string of beads.

After their descent through the oviduct, the eggs of each side of the body form a single string aggregated in a much twisted and tangled mass in the uterus. Considered as individuals without regard to their sequence in the string, the eggs display a striking regularity in their arrangement in the uterus, being packed in parallel spiral rows; but this is merely the result of mechanical pressure, as the string pursues a very sinuous and complicated course throughout the mass.

The egg capsules at the end of the uterus nearest the cloaca, hence those first formed, do not contain eggs; those nearest the oviduct, hence the last formed, are likewise devoid of eggs. These empty egg capsules are in general smaller than those that contain eggs, with a regular gradation in size from those at the extremity of the cord, which are scarcely more than a millimeter in diameter, up to those nearest the egg-containing capsules, where the diameter is only slightly less than normal. However small the size, these capsules are always perfectly formed, with a central spherical space; they are never solid. The 'empty' capsules contain a small amount of coelomic fluid in which are distinguishable under the microscope leucocytes, erythrocytes and yolk corpuscles. A cloudy mass of fluid with the same constituents occurs in the upper part of the uterus, outside of the egg envelopes. Similar capsules devoid of eggs are the 'wind eggs,' known in various vertebrates: birds, reptiles, sharks, chimaeroids.

As a result of experimental studies on the nature of the stimulus which causes the shell to be formed about the hen's egg, Pearl ('09) reached the following conclusions: (a) the stimulus which sets the shell-secreting glands of the fowl's oviduct into activity is mechanical rather than chemical in its nature; (b) the formation of a shell on the hen's egg is brought about by a strictly local reflex, and is not immediately dependent upon the activity of other portions of the reproductive system (nervous impulse or hormone formation). In this connection it is interesting to note

that in *Cryptobranchus* the mechanical stimulus can hardly be the true cause of the formation of the capsule, since capsules are formed when only a small drop of coelomic fluid is present. Moreover it is here observed that coelomic fluid may pass down the oviduct without becoming enclosed in such capsules; on the other hand, every egg is provided with a capsule.

When distended with eggs, the uterus is spindle-shaped, about 10 cm. long, with a transverse diameter of about 4 cm. at its widest part. Its thin walls have a rich blood supply.

Apparently the eggs do not, as a rule, remain long in the uterus before spawning takes place. During the breeding season comparatively few females are found having eggs in the uteri; the majority of the females captured are either spent or with eggs still in place in the ovaries. Eggs taken from the uteri are, in the great majority of cases, capable of artificial fertilization; this subject will be more fully discussed later.

(b). *Oviposition, and nesting habits.* Under strictly natural conditions egg-laying takes place under cover of rocks in the bed of the stream; but in creek aquaria, arranged to afford conditions as natural as possible without too much cover, the process has been repeatedly observed.

Egg-laying begins slowly, a short string of eggs sometimes protruding from the cloaca for several hours before spawning begins in earnest. In the natural habitat, such short strings of eggs are often found in the open. Later, two long strings of eggs proceed slowly from the cloaca, one from each uterus; the majority of the eggs are then deposited more rapidly, in multiple strands, the process requiring less than five minutes. When egg-laying is completed, the strings are usually twisted together in a single tangled mass.

The 'nest' of *Cryptobranchus allegheniensis* has already been described as either a burrow or a natural cavity under a rock which is wholly or partially submerged. The eggs are not fastened in any way, but are protected by this sheltered position from being swept away by the current.

The nests of *Cryptobranchus japonicus* have been described by Ishikawa ('04), and closely resemble those of *C. allegheniensis*.

Das Tier legte seine Eier in tiefe horizontal verlaufende Löcher, in denen das Wasser sehr ruhig ist. Manchmal ist solch ein Loch 10 oder mehr Fuss tief und kaum für das Licht zugänglich. Die Brutstellen für die Eier sind aber nicht immer so tief. Oft fand ich Eier in einem Loch nicht tiefer als 3 oder 4 Fuss. Oeffnet man ein solches Loch, so findet man eine abgerundete Stelle, deren Boden ganz rein gehalten ist.

The nesting habits of *Necturus* have been described by Eycleshymer ('06), and the writer ('11). The eggs are attached singly by their gelatinous envelopes to the under side of a rock, board, or other object lying at the bottom of the water (figs. 55 and 56).

The eggs of *Amphiuma* found by Hay ('88 and '90) in an Arkansas swamp were in a comparatively dry situation, in a small excavation under a log several rods from the nearest water.

Brief reference to the nesting habits of some other amphibia has been made in previous papers (Smith, '06 and '07). Very remarkable are the nesting habits of the anuran *Phyllomedusa*, described by Budgett ('99); the eggs are deposited in a pocket made by bringing together the edges of a leaf overhanging the water.

Amongst the dipnoi, the nest of *Protopterus* (Budgett, '01 a and '01 b) is an oval hole filled with water and surrounded by swampy ground. The nest is at first entirely submerged, but by the partial drying up of the swamp it is left as an isolated pool. *Lepidosiren* (Kerr, '00) nests in a veritable burrow excavated in the black peaty soil of the swamp.

Nesting habits are well known in many teleosts, and in *Amia* (Dean, '96; Reighard '03). According to Budgett ('01 a) the crossopterygian *Polypterus* probably makes no nest, and certainly lays but few eggs at a time, these being scattered broadcast through the thick vegetation of the flooded grass lands. Comparison with *Cryptobranchus* suggests the possibility that these scattered eggs are but preliminary attempts at egg-laying.

(c). *The newly-laid egg and its envelopes.* (Figs. 54, 1 and 2.) In eggs taken from the uterus, the outer egg envelope or capsule fits closely about the egg proper; the envelopes are flaccid and much wrinkled. The capsule of the newly-laid egg takes up water rapidly; in the course of one or two hours a space, filled with fluid, appears between the egg and its capsule, sufficient to enable the egg to orient itself with the animal pole uppermost.

The egg proper is perfectly spherical when fresh, but it gradually becomes slightly oblate from the effects of gravity. It is about the size of a pea, and bright yellow in color—a rather deep yellow at the lower pole, grading to a very pale yellow at the upper. The general intensity of the yellow color varies considerably in eggs of different spawnings, but is quite uniform in eggs from the same female. The absence of black pigment is probably correlated with the fact that the eggs are laid in darkness: the

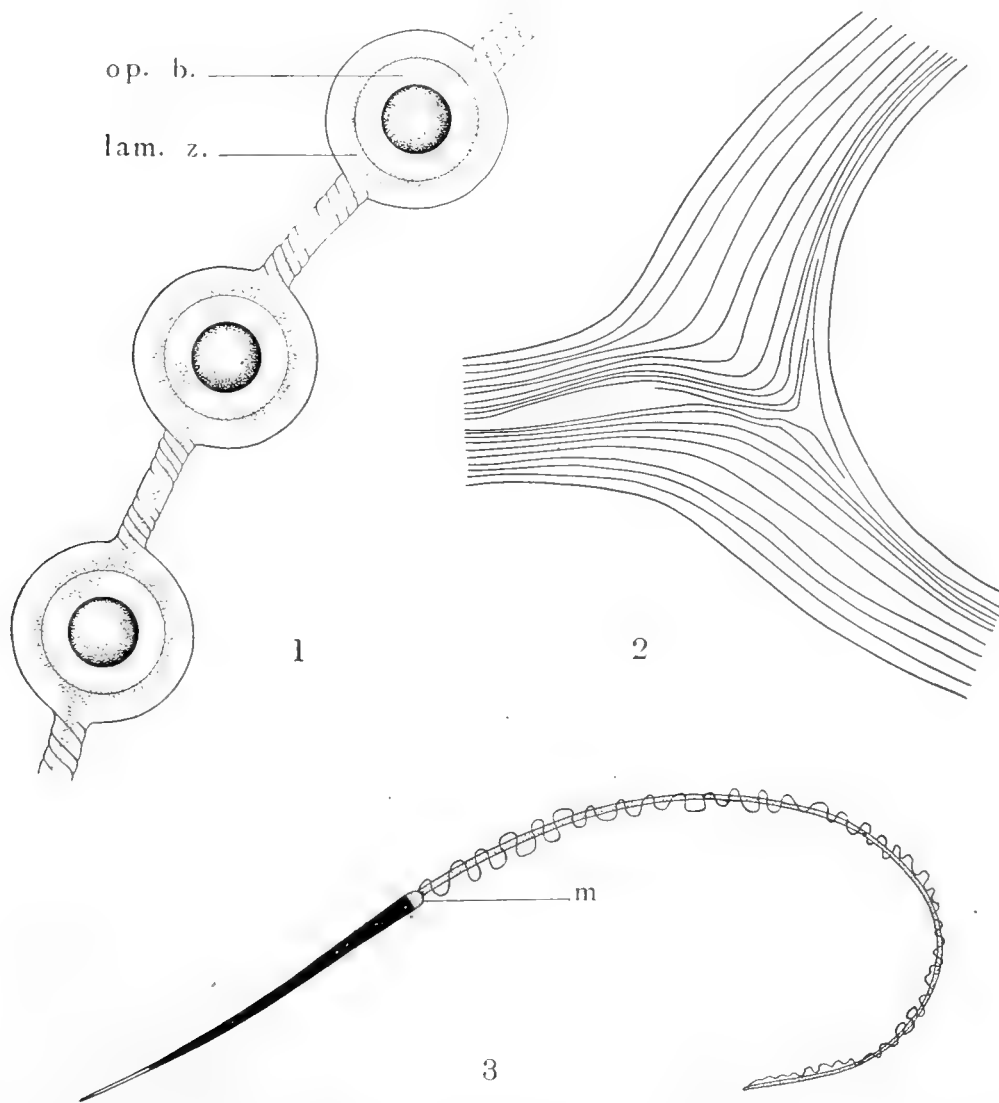


Fig. 1 Eggs and egg envelopes of *Cryptobranchus allegheniensis*, natural size. *op. b.*, opaque body; *lam. z.*, lamellar zone of envelope.

Fig. 2 Optical longitudinal section through the lamellar zone of the envelope in the region of junction of the egg capsule with the connecting cord. $\times 13$.

Fig. 3 Spermatozoon. $\times 500$. *m.*, middle piece.

eggs of *Necturus*, *Plethodon*, *Spelerpes* and *Desmognathus*, which are also laid under cover, are likewise unpigmented.

A very thin and transparent 'vitelline membrane'—the zona pellucida of the ovocyte—closely invests the egg; it is quite inconspicuous in fresh material. This is not the true cell wall of the egg, which, as described in detail on page 112 lies immediately within the vitelline membrane and represents in a modified form the zona radiata of the ovocyte.

Proceeding from within outward, the coverings of the egg may be enumerated as follows: (a) the cell wall; (b) the vitelline membrane lying in close contact with the preceding; and (c) the capsule or thick gelatinous outer envelope, which is separated from the vitelline membrane by a space filled with fluid.

During the first few hours after fertilization the capsule gradually becomes turgid by osmosis, becoming in this way a much more efficient protection to the egg; the space between the egg and its capsule is increased by the absorption of water and in this the egg almost floats, resting lightly on the lower inner surface of the capsule. When the eggs are removed from water the egg proper looks much larger than it really is, because magnified by the spherical capsule.

For a day or two the envelopes are quite soft and somewhat viscous, making it rather difficult to cut them with scissors in order to remove the eggs. Gradually the material of the envelopes becomes firmer. The connecting cord is at first quite elastic, but it loses this quality to a considerable extent after prolonged immersion in water.

Until after the eggs have been in water for several days, the outer layers of the envelopes are still cast into wavy folds or wrinkles, usually extending spirally about the capsules and the connecting cord. As a rule the spiral is constant in the direction in which it extends about the axis of the string in all portions of the cord and capsule. These spiral folds are usually most strongly marked at the ends of the cord adjacent to the capsule, and here they often persist (fig. 1), suggesting the chalazae of the hen's egg.

The envelope is perfectly transparent when fresh, except that wherever viewed tangentially its inner layers have a misty appearance, represented by the shaded zone in fig. 1, and due to a fine lamellar structure sketched in optical section in fig. 2. The misty appearance is caused by the diffusion of light passing through these concentric layers in a direction tangential to their surfaces. The core or axis of the connecting cord has the same misty appearance, due to a continuation of the lamellar structure. The various layers or lamellae of the gelatinous envelope are in intimate contact; there is no fluid-filled space between them such as occurs between the capsule and the vitelline membrane.

The inner layer of the lamellar core of the cord in some cases exhibits a marked twisted or spiral arrangement, like that of the inner portion of the cord connecting the eggs of *Ichthyophis* as described by the Sarasins ('87-'93).

The eggs of a given spawning are fairly uniform in size, but there is considerable variation in the size of eggs from different parents. The average dimensions of the living egg and its envelopes, after two days' immersion in water, are as follows:

Diameter of egg proper.....	6.2 mm.
Diameter of egg with envelope	18 mm.
Diameter of connecting cord.....	5 mm.
Distance of one egg from another, measured from center to center along the cord, about	30 mm.

A few egg capsules, particularly among the empty ones, are double, formed by the union of two capsules without a connecting cord. In such cases the cavities of the two capsules are usually separated only by a thin gelatinous septum; but all gradations occur between this condition and that in which two capsules are connected by an unusually short cord. Rarely, three capsules are closely approximated.

I have found a few instances in which two eggs occurred in the cavity of one simple capsule, without any separation by a gelatinous membrane. It would seem possible that double embryos might be formed in this way, by the fusion of the yolk masses of two such eggs; but this could not account for the only double

embryo that I have found in nature, for in this case, to be described later, each embryo is half the normal size.

After fertilization, numerous spermatozoa are found imbedded in the egg capsule, and floating in the liquid between the capsule and the egg; they also occur in capsules that do not contain eggs. The spermatozoa occur singly, not in masses, and they are entirely absent from eggs taken from the uterus. Fertilization occurs only after the eggs have been deposited in the water (Smith, '07).

An envelope so tough and thick as that of *Cryptobranchus* must exert a decided selective power with regard to the spermatozoa; of a considerable number of spermatozoa simultaneously coming in contact with the envelope, the most vigorous, as well as the ones structurally best adapted, would succeed in first entering the egg.

Floating in the liquid between each egg and its envelope, there occurs a fairly large irregular and slightly opaque mass, in appearance like a faint white cloud (see fig. 1; this mass is also faintly shown in the photograph, fig. 54). Under the microscope it is found to consist of a clear viscous matrix in which are imbedded numerous leucocytes and occasionally a few erythrocytes. In fertilized eggs, this mass, which I have called ('07) the 'opaque body' sometimes contains spermatozoa, but they are not restricted to it, nor especially numerous in it. The opaque body is uniformly present in eggs that do not contain spermatozoa.

A mass similar in general appearance and location to that described above as the opaque body, is figured by Ishikawa ('04) within the egg capsule of *Cryptobranchus japonicus*. In the text he refers to these masses as 'Samenhaufen,' and speaks of the presence of spermatozoa within the egg capsules as evidence of internal fertilization. He considers it improbable that the spermatozoa are able to penetrate the egg capsule, and supposes that they are taken up into the oviduct before the egg capsules are formed.

DeBussy ('04, p. 11) found a mass ('vlokje') of similar appearance within the capsules of unfertilized eggs of *C. japonicus*. Under the microscope he found the mass to consist of a slimy substance containing red blood corpuscles and yolk granules, but

no spermatozoa. He concludes that the presence of spermatozoa is not essential to the formation of the mass, but that they may merely form an element of it; hence that the name 'Samenhaufen' is scarcely justified. This conclusion is in essential agreement with my results on *C. allegheniensis*; it seems therefore that the masses called 'Samenhaufen' in *C. japonicus* by Ishikawa are of the same nature as the 'opaque bodies' of *C. allegheniensis*, and like them of no significance in fertilization. The opaque body apparently consists of coelomic fluid that has escaped into the oviduct.

The egg strings of *Cryptobranchus japonicus* as described by Ishikawa ('04) and deBussy ('04) closely resemble in structure those of *C. allegheniensis*. Both eggs and capsules of the Japanese form are slightly larger; according to Ishikawa the egg proper is about 7 mm. in diameter, and the capsule varies from 20 to 25 mm. in diameter in different spawnings.

The egg capsules of *Amphiuma* as described by Hay ('88 and '90) have the same general structure as those of *Cryptobranchus*. For an opportunity to examine one of Hay's specimens of the embryological material of *Amphiuma*, I am indebted to Prof. C. W. Hargitt, to whom the specimen had been presented by the finder. The egg capsule has a glistening surface like isinglass; it is thinner and apparently tougher, and the connecting cord more slender, than in *Cryptobranchus*. These peculiarities may be due in part to preservation in alcohol, which tends to produce the same condition in the envelopes of *Cryptobranchus*; but my impression is that the egg capsules of *Amphiuma* are better adapted to retain moisture when exposed to the air.

Other amphibians whose egg capsules are fastened together like a string of beads are *Alytes*, *Ichthyophis*, and *Hypogeophis* (Brauer '97).

The general appearance of the egg capsules of *Necturus* is shown in figs. 55 and 56; some further details of structure are shown in fig. 4. There are three layers to the gelatinous envelope: (a) a comparatively thin but very dense inner layer, consisting of several lamellae; (b) a thicker median layer of moderate density, consisting of many lamellae; and (c) a very thick outer layer of

homogeneous material, much less dense than either of the preceding. This outer layer is produced to form the stalk by which the capsule is attached to some solid object. As seen in optical section, the lamellae of the two inner layers have a somewhat sinuous or wavy outline. Leaving the stalk out of account, the entire structure bears a close resemblance to the gelatinous envelopes of the frog's egg. In the early stages of development of the embryo, the dense inner layer of the capsule fits so closely that it is not clearly differentiated from the embryo; this layer is best

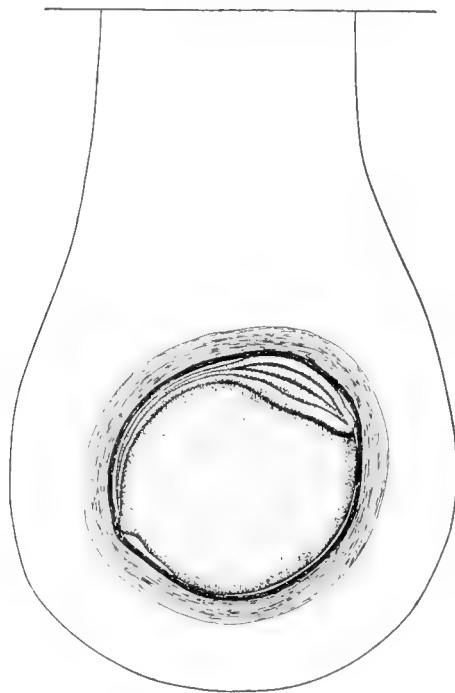


Fig. 4 Optical section through an egg capsule, and surface view of an embryo, of *Necturus*. The embryo is shown in a stage with neural folds, when the capsule is slightly separated from it by a space filled with water. $\times 4$.

studied after the embryo has passed the gastrula stage, when a narrow space, filled with liquid, appears between the embryo and its capsule (see fig. 4). This space is not strictly homologous with the similar space that appears much earlier in the egg of *Cryptobranchus*; for in *Cryptobranchus* the space appears between the gelatinous envelope and the vitelline membrane (*zona pellucida* of the ovarian egg) which remains in close contact with the egg, while in *Necturus* the vitelline membrane apparently func-

tions as the innermost lamella of the capsule. The entire inner layer of the capsule of *Necturus* has a tough consistency similar to that of the vitelline membrane of *Cryptobranchus*; this perhaps is the reason why it is so slow in enlarging.

In *Necturus* the egg proper is slightly smaller than that of *Cryptobranchus*; in the early cleavage stages it measures about 5.8 mm. in diameter.

A general description of the gelatinous envelopes of several species of *Amblystoma* has been given in a previous paper (Smith '11).

5. *The sperm*

The spermatozoon (fig. 3) has been figured by Reese ('04), who fails, however, to picture the middle-piece. The spermatozoon is about 225μ long, and stout in structure as compared with the spermatozoa of *Amblystoma* and *Diemyctylus*. The head, excepting the acrosome, stains deeply with Delafield's haematoxylin. The acrosome appears to be uniformly tapering, not spear-shaped as in *Amphiuma* (described by McGregor, '99). As stated by McGregor ('99) the middle-piece in *Cryptobranchus* is very short in comparison with that of other urodeles. The tail-piece is provided with an undulating membrane, bordered with a convoluted filament.

The ripe spermatozoon is motile, as regards both shaft and filament; but the spermatozoon as a whole is not so flexible as the more slender spermatozoa of *Amblystoma* and *Diemyctylus*. This greater rigidity of the spermatozoon is perhaps correlated with the method of fertilization: the spermatozoon must penetrate the tough and thick egg capsule after a brief exposure of the latter to the hardening effects of water.

In seminal fluid obtained from occasional individuals, an oval mass of granular protoplasm, about 13μ by 16μ , surrounds the posterior part of the head of the spermatozoon. The long axis of this bead-like mass coincides with that of the spermatozoon. I have found a similar mass of protoplasm present in spermatozoa from some individuals of *Amblystoma punctatum*, but here the oval mass usually occurs about the junction of the head and mid-

dle-piece. Probably the condition observed in the two species represents a late developmental stage of the spermatozoon—the metamorphosis of the spermatid into the spermatozoon is not quite complete.

The amount of seminal fluid present at one time in the vasa deferentia of a ripe male is very great in proportion to the size of the animal—a condition correlated, doubtless, with external fertilization. In one instance 20 cc. of seminal fluid was readily stripped from a single male.

6. The method of fertilization

The method of fertilization (external) has already been described (Smith, '07); subsequent observations have supplemented this account only in the fact, discussed later, that a single male may spawn with more than one female.

The method of external fertilization is well adapted to the normal breeding conditions. The 'nest' of *Cryptobranchus* consists of a hollow under rocks, a confined space protected from the current, and filled with very quiet water. As has been shown, the amount of milt that may be discharged at one time by a single male is considerable; in the case of a pair that spawned while being carried in a pail of water, it was sufficient to turn several quarts of water milky white. Such a quantity of sperm set free in the confined space of the nest would become diffused, especially when stirred about by the movements of the animals, so that every egg would be quickly reached and fertilized. As a matter of fact, few unfertilized eggs are found.

So far as I have been able to learn, this is the only case of external fertilization recorded for the urodeles. The inconclusive observations of Kerbert ('04) on *Cryptobranchus japonicus*, and Kunitomo ('10) on *Hynobius*, suggests to me that external fertilization may take place in these forms.

In *Necturus*, from the observations of Kingsbury ('95) it seems certain that internal fertilization takes place. A compound receptaculum seminis or spermatheca is present in the female; spermatozoa have been found in these spermathecae during the

fall and winter, which suggests an autumnal fertilization, though it is possible that spermatozoa are left over from a spring fertilization. The method of transference of the seminal fluid from the male to the seminal receptacle of the female is unknown.

The breeding habits of some urodeles in which internal fertilization takes place by means of spermatophores (e.g., *Amblystoma punctatum* and *Diemyctylus*) have been considered by the writer in former papers (Smith, '07, '10 and '11). Fertilization is external in the anura, internal in the apoda.

In the elasmobranchs and holocephali, fertilization is internal. In the crossopterygian *Polypterus* (Harrington, '99; Kerr, '07 b), during the breeding season the anal fin of the male is modified in such a manner as to suggest internal fertilization; or possibly it serves to direct the sperm against the stream of eggs issuing from the female. Nothing conclusive is known regarding the method of fertilization in the dipnoi. In teleostean fishes, with a few exceptions, fertilization is external; e.g., as in *Chrosomus* (Smith, '08).

The question whether external fertilization in *Cryptobranchus* is primitive or secondarily acquired will be discussed under phylogenetic considerations in a later section.

7. *The brooding habit*

In a previous paper (Smith, '07) a paternal brooding habit was described for *Cryptobranchus*. This was observed in aquaria, and more extensively under natural conditions.

The data on the existence of a paternal brooding habit under natural conditions, while necessarily incomplete, are quite conclusive. In one case, a male occupying a nest containing eggs was observed to fight and drive away several males and a spent female which were attempting to enter the nest (Smith, '07); in another case, a male occupying a nest containing eggs was observed to oppose the attempt of a single male to enter the nest. It is not always possible to tell whether an adult is present in the nest; the rock may be too large to overturn, and while the eggs may be obtained by tilting the rock with a crow-bar, this method

is not always successful in dislodging the adult. In cases where the rock is lifted and overturned, the water is discolored and the hellbender, aided by its protective coloration and the swift current, may escape. When seen, however, it may usually be captured. A record kept for six years ('06-'11) shows that from twenty-nine nests containing eggs a male was captured in ten cases, a female never.

The duration of the brooding habit has not been definitely determined, and perhaps varies greatly. In different nests in which a male was present, eggs were found in various stages of development up to about three weeks old; unfortunately I was obliged to discontinue field work at a date varying from two to four weeks after the beginning of the breeding season. In no case where the eggs were in an advanced stage of development can it be recorded that the male had been continuously present, or even that he was the same male that fertilized the eggs; but the entire absence of females from nests containing eggs is significant.

With regard to the origin of this paternal brooding instinct two suggestions (Smith, '07) were made: (a) the brooding instinct may have originated in connection with the feeding habit; or (b) in holding the nest the male may be primarily concerned in awaiting the coming of another ripe female. Both views assumed that in *Cryptobranchus* we have an example of the brooding habit in an incipient state. Further observations indicate that the brooding habit is well established and manifested as a distinct impulse from the moment of fertilization; its origin is thus thrown back into the remote past, and concerning it we can only speculate.

The evidence for the first interpretation may be briefly stated as follows: Both sexes are voracious eaters of the newly-laid eggs; during the spawning season the majority of the adults taken have the stomach filled with eggs. There is evidence that the females, when opportunity is afforded, gorge themselves with eggs more freely than the males. The number of eggs found in the stomach of a single adult usually ranges from fifteen to twenty-five, a number sometimes greatly exceeded in the stomachs of spent females. In one case, in which the body of a spent female appeared greatly swollen, the stomach was found to be greatly dis-

tended with eggs. When removed and measured by displacement of water, the stomach and its contents were found to have a bulk of over 200 cc. The mouth also was full of eggs, and strings of eggs protruded from the pharyngeal openings. The quantity of eggs present seemed to represent almost an entire spawning. The feat of swallowing such a quantity of eggs would seem possible only if they were taken before the swelling of their envelopes.

The digestive processes of the hellbender are extremely slow, and I have taken undigested eggs from the stomach a week after they were eaten. Under these conditions the presence of a single male hellbender in the nest is in the main protective. On account of the small number of eggs eaten at once, and the slowness of his digestive processes, fewer eggs are eaten than would be the case if other hellbenders, and especially the spent females, had free access to the nest.

As previously noted, a male has been observed to fight and drive away a spent female and several males that were attempting to enter the nest. The male in such cases has the advantage over the female because of the weakened condition of the latter; as regards the other males, he has the advantage of position.

The facts suggest that the male, in thus driving away others of his own kind, may be primarily concerned in guarding his own food supply; this guarding habit may become modified into a true brooding instinct. But it is difficult to believe that the male, after having filled his stomach with eggs, would any longer be concerned with the fate of the remaining eggs on account of their value as food.

According to the second interpretation, the male may hold the nest in expectation of the coming of another ripe female; an extension of this habit may give rise to the brooding instinct. According to this view, the brooding instinct has its origin in the breeding habit.

The reception of a ripe female by a male guarding eggs has not been directly observed, but the following data afford sufficient evidence on this subject: Out of twenty-nine nests examined during the seasons of 1906–1911 inclusive, eleven were found to contain eggs of at least two different spawnings, the product of

different females. The eggs were in different stages of development, hence fertilized at different times. In two cases the number of eggs present in a single nest was sufficient to represent at least three spawnings by different females. It is possible to determine this with considerable certainty, for the number of eggs matured by a single female each season is limited, and these are all laid at one time. Moreover it is known that the intensity of the yellow color of the eggs is constant for all the eggs of a single female, but varies considerably in eggs from different individuals. In view of the observed vigilance and effectiveness of the male in possession of the nest in driving away other males, it is highly improbable that successive pairs of adults have occupied the nest; hence the facts indicate that the same male has spawned with successive females.

The second hypothesis seems supported by better evidence than the first; but while it is entirely possible that such may have been the origin of the habit in the remote past, there is evidence that at present the eggs are the object of paternal care from the time of fertilization, and this brooding instinct is only temporarily overcome by hunger or diverted by the breeding instinct. The behavior of males breeding in aquaria strongly suggests this: after fertilizing the eggs the male usually remains close beside them or crawls under or amongst them.

Concerning the brooding behavior of some specimens of *Cryptobranchus japonicus* in captivity Kerbert ('04) says:

Nach Beendigung der Eiablage legte sich das Weibchen offenbar in grösster Ermattung in eine Ecke des Behälters hin und kümmerte sich um das Gelege gar nicht mehr. Das Männchen hingegen hat seitdem die Eiermasse nicht verlassen—ja sogar *die Brut fortwährend bewacht*. Denn sobald das Weibchen die Eiermasse zu nahe kam, stürzte das Männchen in sichtbarer Wut auf die Mutter los und vertrieb sie. kriecht der Männliche Riesensalamander zwischen den verschiedenen Strangen der Eiermasse hindurch und bleibt dann von der Eiermasse umhüllt liegen, oder er legt sich einfach neben die Eiermasse hin. In beiden Fällen aber hält er, hauptsächlich durch eine pendelartige Bewegung des ganzen Körpers, von Zeit zu Zeit die ganze Eiermasse in Bewegung. Durch diese Bewegung entsteht eine für den Atmungsprozess der Eier und Embryonen höchst wichtige Wasserströmung, während die Lage der Eiermasse hierdurch gleichzeitig fortwährend wechselt.

It thus appears that the paternal brooding instinct in both species of *Cryptobranchus* is manifested from the moment the eggs are fertilized, though in *C. allegheniensis* at least it may be temporarily inhibited or overcome by hunger or by the breeding instinct. The brooding habit of *Cryptobranchus* is undoubtedly very old, and we must look to other forms to find examples of it in the incipient condition.

According to Whitman ('98) there are three distinct elements in brooding behavior: (*a*) the disposition to remain with or over the eggs; (*b*) the disposition to resist and to drive away enemies; and (*c*) periodicity. The first of these elements has its origin in the need for rest, protection to the offspring being at first incidental. The second element, pugnacity, is periodical and a part of the reproductive cycle. The third element, periodicity, is apparently an attribute of the two other elements, based on physiological conditions; its adaptiveness lies in correlating the other two elements with the hatching period of the eggs.

In *Cryptobranchus*, after spawning, the female is evidently much the weaker of the two; as a matter of observed fact, she is driven away by the stronger and more pugnacious male. It can scarcely be the need for rest that keeps the male in the nest, since he maintains exclusive possession at the cost of alert watchfulness and occasional combat. If the element of weakness were the important factor in initiating the brooding habit, we should expect the female rather than the male to remain in the nest. It may be that primitively the brooding impulse is a phase of the reproductive cycle that applies to both sexes, the female losing it on account of her hungry and exhausted condition due to the accumulation of a large amount of yolk in the egg. Perhaps (for this suggestion I am indebted to Professor S. J. Holmes) on the part of the male there is involved a proprietary interest in the nest, which he has chosen and in part excavated, and which he occupies as an advantageous breeding place and as a more or less permanent home.

To obtain conclusive evidence regarding the origin of the brooding habit one must study a series of closely related forms illustrating the habit in the making. In *Cryptobranchus* it appears

that the habit is well established; it is improbable that the question can be settled by the study of this form alone, and the data here given are presented only in the hope that they may contribute something toward the final solution of the problem.

Concerning the brooding habit of *C. japonicus* in its natural habitat Ishikawa ('04) says: "Fast in jedem Loch, wo man von Ende August bis zu Anfang October ein weibliches Tier gefunden hat, findet man einen Eiklumpen. Dieser Umstand lässt schon vermuthen, dass das Tier eine Brutpflege hat wie *Ichthyophis* oder wie so viele andere Amphibien." Kerbert, however, asserts ('04) that it is the male that guards the eggs, and states that the sex of his specimens was carefully determined.

Other amphibia known to possess brooding habits are the urodeles *Desmognathus* and *Plethodon*; the caecilians *Ichthyophis* and *Hypogeophis*; *Alytes* and several other anura (Wiedersheim '00). In the cases of *Desmognathus*, *Plethodon*, *Ichthyophis* and *Hypogeophis* the female is said to care for the eggs; in the case of *Alytes*, the male.

The brooding habit seems to be lacking in *Necturus*. According to Eycleshymer ('06), *Necturus* sometimes eats the eggs of its own species.

The brooding habit is well known in many teleosts, and in *Amia* (Dean, '96; Reighard, '03); it is well developed in the lungfishes *Protopterus* (Budgett, '01 a and '01 b), and *Lepidosiren* (Kerr, '00). I can find no record of any observations pointing to the existence of a brooding habit in the *crossopterygii*.

D. SUMMARY

The breeding season of *Cryptobranchus allegheniensis* in northwestern Pennsylvania begins about the first of September and lasts about two weeks.

There is a tendency toward segregation of the females from the breeding grounds during a period extending from the close of the breeding season until the middle of the following summer.

About 450 eggs are matured each year by an adult female of average size. The egg capsules from each oviduct are fastened

together in a single string. At the ends of each string are formed some small but perfect capsules which do not contain eggs.

The eggs are unpigmented, heavily yolk-laden and strongly telolecithal.

The nest consists of a submerged cavity under a rock in the bed of the stream. The cavity is sometimes in part the work of the animal.

Fertilization is external.

There is a paternal brooding habit, which is manifested from the moment of fertilization. The origin of this habit is problematical.

III. METHODS AND TECHNIQUE

A. COLLECTION AND CARE OF LIVING MATERIAL

To insure a convenient supply of adults for various purposes, these were collected before and during the breeding season and placed in a large creek aquarium, constructed of wire netting and placed in shallow water with a gentle current. This arrangement of the aquarium afforded abundant aëration; flat stones placed on the bottom provided cover; in general the conditions closely resembled those of the natural environment. The aquarium proved of great value as a means of insuring a supply of adults for use at frequent intervals in securing material for the study of ovogenesis, maturation, fertilization and the early cleavage stages.

Artificial fertilization was often resorted to in order to control the time of fertilization for the study of fertilization and early cleavage stages, and occasionally eggs were used that had been deposited and fertilized by specimens in captivity; but the greater part of the material used for the study of the development was obtained from the nests of the animals in their natural environment.

At first the problem of keeping the eggs alive in a favorable environment while studying their development promised some difficulty. Early attempts to keep the eggs in creek aquaria met with disastrous failure through the attacks of water-mould. The method finally employed was to keep the eggs in shallow

earthenware dishes containing well water, in a cool cellar; a limited number of eggs were placed in each dish, and the water changed daily. Under these conditions they developed normally. During the early autumn all the laboratory work on the living egg, and the preservation of material, were carried on in this cellar, so that at no time were the eggs subjected to an unfavorable temperature. The eggs were in general shielded from the light; but for working purposes both direct and diffused sunlight, or a Welsbach light, were used.

On account of teaching duties observations in the field have never extended quite to the time of hatching, consequently it has been necessary to transport the living embryo for considerable distances. In the case of embryos taken after the closure of the neural folds, material shipped in cool weather by express, in a pail containing shallow water, did quite as well as material which was given personal care during transportation and for which the temperature was regulated with ice; in both cases the embryos developed normally. Younger embryos require much greater care in transportation; material in cleavage and gastrula stages shipped by express has usually died or developed abnormally, perhaps in the main because of untimely warm weather; all such material was discarded. Material kept in the laboratory thrives in shallow dishes containing well water, the dishes being partly immersed in cool running water; no artificial aëration is necessary. As a check on possible abnormalities in material that has been transported, I have had a series of late stages preserved from material kept without transportation.

B. FIXATION AND PRESERVATION OF MATERIAL

The envelopes may be removed in any stage without much difficulty, by means of scissors. This is very easily done after the eggs have been in water for several days, since the envelopes become inflated. For earlier stages, more care is necessary. Eggs from the uterus, and fertilization stages, may be handled more rapidly by fixing in Solution B (see below) before the removal of the envelopes; they may be preserved thus in formalin, but not in alcohol. After fixation the envelopes become brittle

and may readily be removed with needles. Comparison with eggs fixed after the removal of the envelopes shows no essential difference in the results.

The fixation of such large and heavily yolk-laden holoblastic eggs presented a problem of considerable difficulty. A great variety of the usual fixing fluids were tried, but none of them succeeded without modification. After extensive experimentation, the mixture described below as Solution B was found to be very satisfactory for all the yolk-laden stages, for surface study, photography and for sectioning.

The following fixing solutions were found useful for the purposes indicated:

Solution A. Formalin, 10 per cent. Useful for preserving eggs in the envelopes for demonstration purposes, or for the study of the envelopes, as it leaves the envelopes clear and preserves the eggs in their natural color. Formalin is of some value for the surface study of cleavage, as it brings out the faint cleavage furrows of the lower hemisphere with great distinctness, and occasionally gives remarkably good preparations for the surface study of the cleavage of the upper hemisphere. In general the fixation of the micromeres is unsatisfactory, both for surface study and for sectioning. Formalin is unsurpassed for fixing larvae for museum purposes; for permanent preservation they should be changed to alcohol.

Solution B. Bichromate-acetic-formalin. The following proportions must be quite strictly adhered to:

Potassium bichromate.....	1 gram
Glacial acetic acid.....	2½ cc.
Schering's formalin, added at the time of using.....	5 cc.
Water.....	92 cc.

Fix about forty-eight hours in plenty of the solution, at a low temperature; change the solution once or twice.

Rinse in water and wash in 5 per cent formalin, in the dark, for at least two weeks, changing the formalin as often as it becomes discolored; preserve in 5 per cent formalin. Preservation in alcohol also gives good results for sectioning, but is not so good for surface study nor for photography.

During the process of washing in formalin the color changes from yellow to green. The yolk becomes dark green, while the blastodisc or embryo proper is much lighter in color, giving a sharp differentiation of the protoplasmic portions of the egg. The form of the egg is preserved perfectly, and remarkably good definition for surface study is secured. The eggs are easily sectioned by the paraffin method.

Not until after the closure of the neural folds is it possible to alter the proportions in the formula as given above without injury to the form of the embryo; an increase in the proportion of potassium bichromate results in the collapse of the embryo when in melted paraffin if not in an earlier stage of the process of preparation for imbedding. For later stages the proportion of potassium bichromate may be slightly increased (e.g., to 1½ per cent), without detriment to the surface features and perhaps with some gain in the histological results.

Solution C. Sublimate-acetic-formalin.

Saturated solution corrosive sublimate in 10 per cent formalin . . .	97½ parts
Glacial acetic acid	2½ parts

Fix for a few hours, then transfer to formalin for a few days to insure thorough fixation of the yolk. Wash and preserve in either formalin or alcohol.

This is not so satisfactory a fixing solution as Solution B, but may be used for comparison. For surface study the results, especially in the early stages, are decidedly inferior to those secured with Solution B. For sectioning, good results are secured in the early cleavage stages and after the closure of the neural folds; the mercury crystals must be removed by prolonged treatment with iodine. In the blastula and gastrula stages the embryo usually collapses during the process of preparing for imbedding.

Solution D: Lavdowsky's.

Formalin	10 parts
Alcohol, 95 per cent	50 parts
Glacial acetic acid	2 parts
Water	40 parts

Fix for several days; preserve in 70 per cent or 80 per cent alcohol.

This mixture is especially useful for the yolk-laden ovarian eggs, and for maturation stages; it is not very satisfactory for embryonic stages. Envelopes, if present, must be removed before the eggs are fixed in this solution. The best results are obtained by sectioning the material soon after preservation.

Solution E: Zenker's. This mixture was found most useful for the early stages of ovogenesis, before the formation of any considerable amount of yolk. It is not good for embryonic stages, unless parts of the embryo are to be dissected off from the yolk before sectioning. It gives very inferior preparations for surface study in every stage.

For the early stages of ovogenesis, before the formation of yolk, both Flemming's and Bouin's solutions were used with fair results. For larvae after the disappearance of the yolk sac, Tellyesnick's, Zenker's, or almost any good fixing solution may be used.

Of the various mixtures experimented with for the yolk-laden stages, those containing picric acid proved to be the very worst. The invariable result of the use of a solution containing picric acid was to cause the egg to disintegrate.

In preserving the embryological material of *Necturus*, Solution B was principally used. In the early stages of development, before the formation of the neural folds, the results are not so uniformly good as with *Cryptobranchus*; this is perhaps due to the fact that in these stages the eggs of *Necturus* are almost necessarily preserved before the removal of the very closely-fitting gelatinous envelopes. In successfully preserved eggs in the cleavage stages, the furrows of the upper hemisphere are more conspicuous and the contour of the micromeres more rounded, than in *Cryptobranchus*; they are thus, except for difficulties arising from the character of the envelopes, more favorable objects for photography. Late gastrula and neural groove stages of *Necturus*, preserved by this method, are rarely so favorable for surface study as the same stages in *Cryptobranchus*. After the formation of the neural folds, when a space has appeared between the envelope and the egg, the embryos of *Necturus* are preserved

with very uniform success, whether fixed before or after the removal of their envelopes. In particular, stages after the closure of the neural folds give a sharpness of detail in the surface features rarely found in *Cryptobranchus*; these stages of *Necturus* are very favorable objects for photography.

C. SECTIONING AND STAINING

Kerr ('01), in describing the technique employed in studying the egg of *Lepidosiren*, has well said: "The investigation of a holoblastic egg 7 mm. in diameter and packed with yolk involves great technical difficulties, for the whole of each egg has to be converted into thin sections. The full extent of these difficulties will only be appreciated by embryologists who have essayed a similar task." In sectioning the heavily yolk-laden stages, Kerr used the celloidin method, and a combination of the celloidin and paraffin methods. DeBussy ('04) used the celloidin method in studying the cleavage stages of *Cryptobranchus japonicus*.

For sectioning the embryological material of *Cryptobranchus allegheniensis* and *Necturus* I have used the paraffin method exclusively; success with this method was found to be entirely a matter of careful attention to technique. The most important considerations are proper fixation and washing, and thorough infiltration with paraffin. In handling serial sections of large numbers of these eggs the advantage of the paraffin method is obvious.

With regard to staining, for general purposes the best results were obtained by staining *in toto* with Grenacher's borax carmine, and counterstaining on the slide with Lyons blue in absolute alcohol; to the Lyons blue solution sufficient picric acid was added to turn it green. By this method the effect of a triple stain, with excellent differentiation, is obtained. The chromatin is stained red, cell walls and cytoplasm blue; the yolk is first stained red by the borax carmine, but turns green in the counterstain. It is usually best to cut short the action of the counterstain at a time when the smaller yolk particles are stained green, while the larger ones are left red. The method has the advantage of

rapidity, an important consideration when great series of large sections are to be handled in considerable numbers.

In sectioning and staining the early cleavage stages the exact mode of procedure is as follows:

From formalin pass the eggs to alcohol, 35 per cent, 50 per cent, two hours each.
Grenacher's borax carmine in 70 per cent alcohol, about two days.

Acid alcohol (0.25 per cent HCl in 70 per cent alcohol), about two hours.

Ninety-five per cent alcohol, two to twelve hours; 100 per cent alcohol, two to three hours.

Xylol, four to ten hours.

Paraffin with melting point 52° C. (at a temperature not exceeding 55° C.), two days. Change the paraffin at least once.

Imbed in a paper box, hardening the block under alcohol.

Cut sections 10 μ to 15 μ thick, using a Minot rotary microtome.

Counterstain on the slide with Lyons blue and picric acid mixture in absolute alcohol.

Wash in xylol long enough to destain slightly.

Mount in Canada balsam.

Early stages require longer for fluids (especially paraffin) to penetrate than do later stages. For the yolk-laden ovarian eggs, and maturation and fertilization stages, from two to three days in borax carmine, and about three days in melted paraffin, are necessary. I have found no serious ill effects in these stages from this prolonged immersion in paraffin at the temperature given.

Material fixed in Lavdowsky's solution stains and infiltrates more rapidly than with the other methods of fixation; also the yolk is less likely to crumble.

Late cleavage, and gastrula stages, are penetrated by the various fluids more rapidly than the early cleavage stages, so that the time may be reduced to two-thirds or one half. For still later stages, there is a further gradual reduction in the length of time required.

During the present year, at the suggestion of Professor Wilson, I have employed a slight modification of the method described above. After being cleared in xylol, the objects were left several days in a mixture of xylol and paraffin at about 38° C. By this preliminary treatment, the time required for infiltration with melted paraffin at a high temperature was reduced at least one-half, with some improvement in the quality of the preparations.

IV. THE EXTERNAL HISTORY OF THE EGG BEFORE CLEAVAGE

Some superficial aspects of the history of the egg before cleavage have already been considered in connection with the account of the breeding habits.

A. EXTERNAL CHANGES PRECEDING AND ACCOMPANYING MATURATION

Except where otherwise mentioned, the observations recorded under this heading were made on the living egg.

If the ovary of an adult *Cryptobranchus* be examined at any time during the summer, the eggs which are about to become mature are readily distinguishable by their much greater size and yolk content.

In the living ovaries of adults taken about the middle of August, the eggs show no positive surface indications of a telolecithal structure. The same eggs fixed by a variety of methods show a circular area or 'calotte' about 60° in diameter, which is somewhat lighter in color than the remaining surface of the egg. On account of its large size the egg now causes the ovarian wall to bulge strongly outward. In general the pale circular area is situated in the center of the more exposed hemisphere of the egg, and is not so profusely covered with ovarian blood-vessels as the remainder of this hemisphere, but this relation is not always exact.

Sections show that the calotte is the outward expression of a peripheral disc-shaped region richer in protoplasm and small yolk granules than the remainder of the egg; in the center of this disc lies the germinal vesicle. From its homologue in the teleostean egg I shall call this region the *germinal disc* or *blastodisc*; in surface views it may be referred to by the same names, or more strictly speaking, as the *germinal area*. Fixation serves to accentuate the optical differences between the germinal disc and the remainder of the egg, making the germinal area visible in preserved material at an earlier stage than in the living egg. The center of the germinal area defines the animal pole of the egg.

Shortly before the egg is ready to leave the ovary, the germinal vesicle appears at the very surface, at the center of the germinal

area which is now visible in the living egg. After remaining here for a length of time that has not been accurately determined, the germinal vesicle disappears from view leaving only a faint dark spot to mark its former site.

In most ovaries obtained about the time of the beginning of the breeding season (the last week in August and the first week in September), all stages in the emergence of the germinal vesicle will be found; in some eggs the germinal vesicle has not yet reached the surface, but in a considerable proportion of cases it will be found exposed in varying degrees (see fig. 53).

The phenomena concerned with the appearance of the germinal vesicle at the surface are very striking, owing to the large size of the germinal vesicle, the sharp contrast between its transparent fluid contents and the surrounding opaque substance of the egg, and the distinct appearance of several opaque-white bodies, presumably nucleoli, within the germinal vesicle. All this may be seen even with the naked eye.

In order to obtain the sequence of the changes occurring in a single egg during this stage, many individual eggs were isolated in normal salt solution, or identified while in position in the ovary, and kept under observation for several hours. In the case of the first ovary studied during the fall of 1907, some of these eggs changed sufficiently before death ensued, to enable me, by combining several individual histories, to get a fairly complete idea of the normal course of events in a given egg. But in succeeding years, although several dozen ovaries containing eggs in this stage have been studied in females recently killed or anaesthetized with chlorotone, no marked changes could be detected. Hence in the following account dependence is placed chiefly on a comparison of individual eggs in the same freshly-exposed ovary, and a comparison of ovaries in slightly different stages of development. In particular, incipient stages in the approach of the germinal vesicle to the surface could be distinguished from possible later stages in which it has disappeared from view, through a comparison of ovaries such as those described above, with others in which nearly all the eggs had been set free from the ovary.

The first indication of the approach of the germinal vesicle to the surface is the appearance of a faint dark spot, 1 to 2 mm. in diameter, in the center of the blastodisc. This dark spot grows more distinct; it is the optical effect of the scarcely-submerged germinal vesicle. Within this large dark area appears a small sharply defined much darker area, circular in outline, which grows at the expense of the larger and fainter dark area. At the time of its first appearance the small dark area is sometimes seen to pulsate slowly, quiver and change form, disappear and reappear. This small dark spot is a portion of the germinal vesicle which is actually in contact with the zona radiata (see section V). It may increase in size until almost an entire hemisphere of the germinal vesicle is exposed. In light of moderate intensity the germinal vesicle appears as a deep, dark well of transparent substance walled in by the opaque material of the blastodisc; in strong sunlight one may see within the germinal vesicle the reflection of the bright yellow yolk beneath. Several opaque-white bodies of various sizes appear within the germinal vesicle; these are probably nucleoli, though the largest ones are much larger than the nucleoli shown in sections.

An ovarian egg dissected out and immersed in water at the time of the appearance of the germinal vesicle at the surface orients itself with the animal pole upward.

The actual disappearance of the germinal vesicle from the surface, and the relation of this process to the rupture of the nuclear wall, have not been satisfactorily observed. Whether the germinal vesicle recedes slightly from the surface before or during the rupture of its wall, or disintegrates at the very surface, has not been positively established; it is possible that all three conditions occur in different eggs. In several cases there seemed to be a welling-up of material from the germinal vesicle which spread out to form a broad crater at the surface; in other cases the appearances favored the impression of a slight subsidence of the germinal vesicle. It is possible that the egg has never been observed at the exact time of the rupture of the nuclear wall, for though a large number of eggs from ovaries containing eggs with

the germinal vesicle at the surface have been sectioned, in none of these eggs has the nuclear wall been found ruptured.

Several females have been taken in which only a few eggs remained in the ovary, the others being found in the body cavity, oviduct and uterus. The ovarian eggs of such specimens were invariably found to be in a later stage than those just described: sections showed that the dissolution of the germinal vesicle was complete, and in surface views these eggs showed a small faint dark spot or slight depression at the animal pole (see fig. 5).

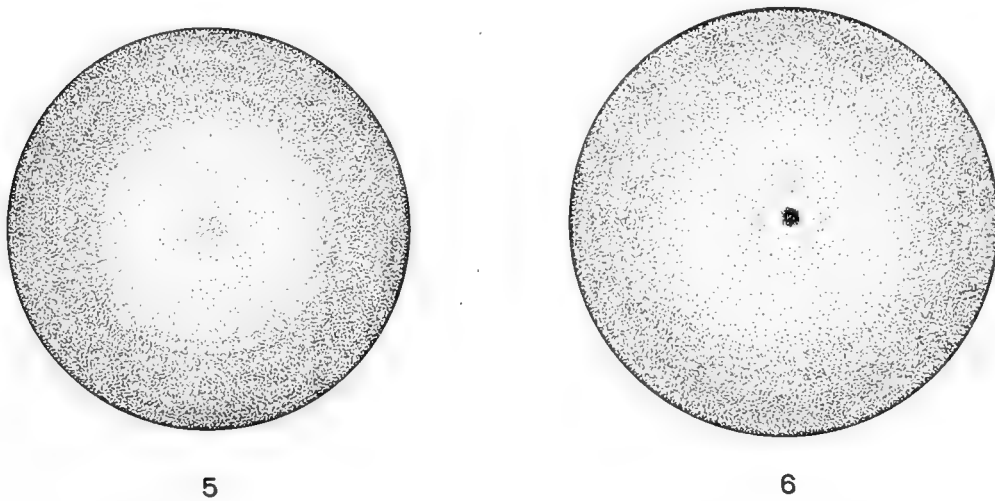


Fig. 5 Surface view of the animal hemisphere of an egg of *Cryptobranchus allegheniensis* ready to leave the ovary, after the rupture of the germinal vesicle. The lightly stippled area indicates the blastodisc. Sketched from the living egg. $\times 7$.

Fig. 6 Surface view of the animal hemisphere of an egg taken from the uterus, ready for fertilization, showing pit at the center of the blastodisc. Sketched from preserved material. $\times 7$.

The dark spot is sometimes surrounded by a tumid ring, but this condition is probably pathological.

At the time of the escape of the egg from the ovary and its passage through the body cavity and upper oviduct, the egg seems softer in consistency than at other times. Some fixing solutions, particularly Lavdowsky's, which usually preserve perfectly the spherical form of the egg, now fix it as an irregularly-shaped mass. This plasticity may be of use to the egg in its escape from the ovary and passage down the oviduct.

In eggs taken from the lower oviduct there is found a slight extension of the blastodisc and a marked increase in the intensity of its differentiation, both in living and preserved material. Moreover, outside the rather indefinite limits of the blastodisc proper there seems to be a continuation of the same sort of material as an extremely thin whitish superficial layer extending beyond the equator and well into the lower hemisphere.

In eggs entering the uterus the blastodisc is well differentiated throughout an area about 90° in diameter, while the entire remaining surface of the egg shows a slight paleness as compared with earlier stages.

The dark spot or shallow depression at the animal pole persists, though often very faintly, up to about the time of fertilization, when its site is occupied by a minute but deep and sharply-defined pit (see fig. 6). The change usually does not take place until after the eggs have been for some time in the uterus. As shown by the study of sections, the appearance of this pit usually coincides with the time of formation of the second polar spindle.

B. CAPACITY OF UTERINE EGGS FOR FERTILIZATION

To test whether eggs newly arrived in the uterus are capable of fertilization, a female was taken in which only a small portion of the eggs had reached the uteri, the others being distributed all along the route from ovary to uterus. The eggs from one uterus—about 75 in number—were mixed with milt after the usual manner in artificial fertilization. Of the entire lot, not a single egg developed.

In another female nearly all the eggs had arrived in the uteri, a few remaining in the oviducts and body cavity, and none in the ovaries. All the eggs from the uteri were mixed with milt; about 5 per cent of them developed.

In a third female all the eggs were in the uteri, but none of them showed a distinct pit at the animal pole—evidence that they had only recently entered the uterus. All the eggs were mixed with milt; none of them developed. It should be noted that this female was evidently in the first year of sexual maturity and the

eggs may have been slow in undergoing maturation changes, or defective in some way.

In the great majority of cases of females taken with all the eggs in the uteri, artificial fertilization has been successfully performed; a high percentage of fertilized eggs is reached when all the eggs show a distinct pit at the animal pole. In every case in which seminal fluid was examined under the microscope during the breeding season, the spermatozoa were motile; so it is not likely that any cases of failure in artificial fertilization were due to defective spermatozoa.

The evidence indicates that the eggs are incapable of fertilization at the time when the first eggs reach the uterus, but that about the time all the eggs reach the uterus the majority of them become capable of fertilization. This change in their potentiality coincides in time with, or slightly precedes, the formation of a distinct pit at the animal pole; it is probably correlated with the formation of the second polar spindle (see section V).

C. CHANGES VISIBLE FROM THE SURFACE DURING FERTILIZATION

The appearance of the blastodisc shortly after fertilization is shown in figs. 7 and 8. During the first eight hours after fertilization there is an increase in the extent of the blastodisc from a diameter of 90° to 130° – 160° , with a corresponding increase in the intensity of its differentiation. From this time up to first cleavage there is no constant increase in the extent of the blastodisc, though the transition from the blastodisc to the darker region surrounding the vegetal pole becomes more gradual. The pit at the animal pole persists unchanged almost up to the time of first cleavage; it is sometimes double (see fig. 10). Shortly before first cleavage it becomes broader and shallower, and usually disappears before the beginning of the first cleavage furrow.

As early as fifteen minutes after artificial fertilization, pits or scars made by the actual or attempted entrance of a spermatozoon have been found on the surface of the egg. It seems remarkable that the spermatozoon can pierce through the thick and tough gelatinous capsule in so short a time. In living material, the

point of actual or attempted entrance of a spermatozoon is often visible as a minute but sharply-defined pit, barely visible to the naked eye; hence the name 'sperm pit' will be used to designate the precise locality where the spermatozoon enters, though the word 'pit' does not always accurately describe the appearance in preserved material.

The sperm pits are best studied in material killed in the bichromate-acetic-formalin mixture and preserved in formalin. The 'pits' are not all alike, but readily fall into the following classes, which probably represent consecutive stages in the penetration of the egg by the spermatozoon (see fig. 8):

(a). A simple pit, deep and sharply defined, as observed in living material.

(b). The pit is surrounded by a very small circular opaque white spot.

(c). The pit has disappeared, and the white spot remains. This type is most numerous. (Rarely, the pit persists until much later—see fig. 10.)

(d). The white spot is surrounded and sharply limited by a dark circular line.

(e). The white spot is surrounded by two concentric circular lines separated by a narrow space which is darker than the general surface of the egg (best shown in fig. 7).

It is not always possible to tell from surface views whether the spermatozoon has actually entered the egg, but from the study of

* Fig. 7 Equatorial view of an egg of *Cryptobranchus alleghehiensis*, 15 minutes after fertilization, showing a single sperm pit. The lightly stippled area in the upper part of the figure indicates the extent of the blastodisc.

Fig. 8 Equatorial view of an egg 45 minutes after fertilization, showing numerous sperm pits.

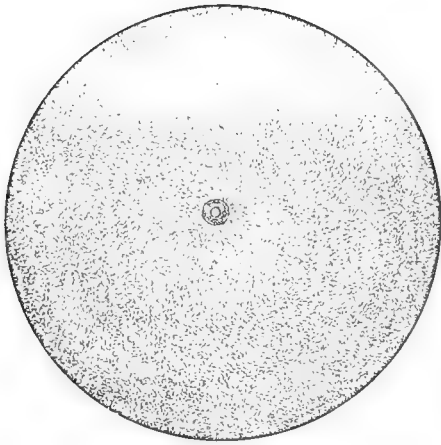
Fig. 9 View of the animal hemisphere of an egg $3\frac{3}{4}$ hours after fertilization, showing a sperm area near the edge of the blastodisc.

Fig. 10 View of the animal hemisphere of an egg $3\frac{3}{4}$ hours after fertilization, showing a later stage in the history of the sperm area. The boundary of the sperm area is a trifle too conspicuous in the figure.

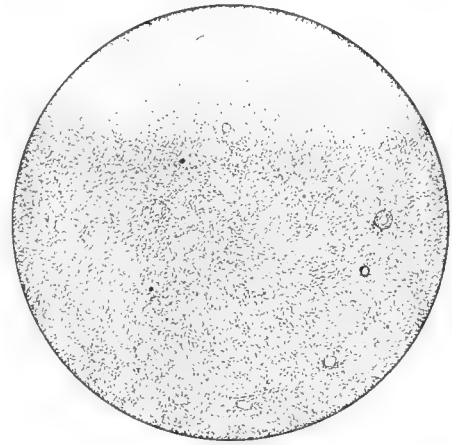
Fig. 11 Equatorial view of an egg $6\frac{1}{2}$ hours after fertilization, showing further extension of the sperm area.

Fig. 12 View of the animal hemisphere of an egg $7\frac{1}{2}$ hours after fertilization, showing two sperm areas, on opposite sides of the blastodisc.

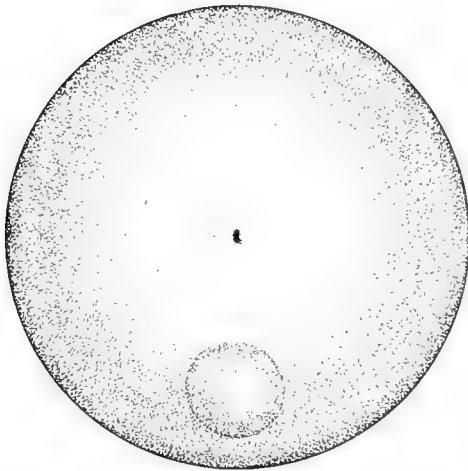
All the figures are drawn from preserved material. $\times 7$.



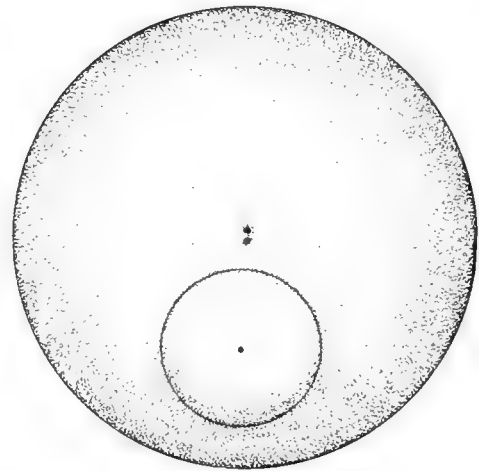
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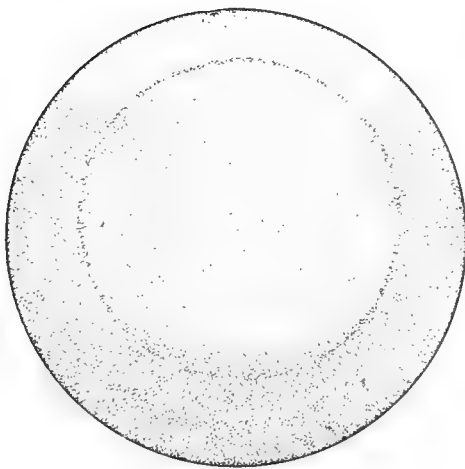
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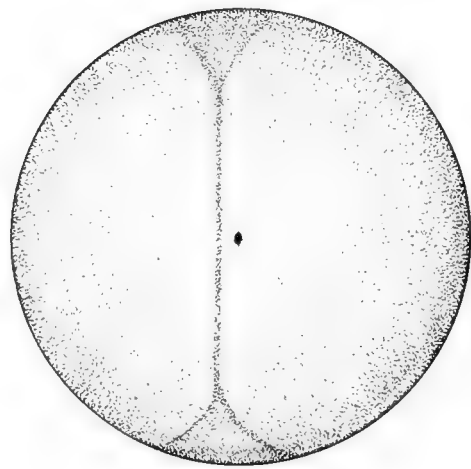
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sections it appears that the first three types of sperm pits indicate that the spermatozoon has barely penetrated through the cell wall, or that the attempt is an abortive one; the last two types indicate with considerable certainty that the spermatozoon has penetrated well into the egg.

Polyspermy is the rule. Cases of penetration by more than one spermatozoon have been found fifteen minutes after fertilization, while the surface of the egg may be scarred by a dozen or more wounds presumably made by other spermatozoa. An hour later, the majority of the eggs have been penetrated each by from one to ten spermatozoa, and sometimes scarred by as many as fifty more. In one case observed the entire number of sperm pits reached nearly a hundred.

About three hours after fertilization the small white spot representing the sperm pit is surrounded by a circular area about 10° to 15° in diameter, slightly darker than the general surface (see fig. 9). An hour later this area has increased in size, is whiter throughout its central portion, and is sharply bounded by a dark line which forms a perfect circle (see fig. 10). This dark line is, partly at least, due to a slight depression in the general surface of the egg. For convenience the area enclosed by this circle will be called the 'sperm area.'

During the next few hours the sperm area increases in size until it covers almost an entire hemisphere (figs. 11 and 12). Its surface is now in general a trifle paler than the remainder of the egg outside the blastodisc; its boundary may pass the animal pole without interruption. Two or even three sperm areas in this advanced stage may be present, their boundaries usually overlapping. Fig. 12 shows an egg fertilized from two opposite sides, the spermatozoa entering near the margin of the blastodisc; the two sperm areas meet at the animal pole, but remain widely separated in the lower hemisphere.

In monospermic eggs preserved and dissected in this stage, the sperm area is found to overlies a lenticular or disc-shaped mass, of firmer consistency than the remainder of the egg which may sometimes be shelled off in a few concentric layers like the fleshy part of an onion. Outside the boundaries of both sperm

area and blastodisc there is left a crescentic area which retains the usual color of the heavily yolk-laden portions of the egg; this region often shows numerous fissures in the yolk, running parallel to the margin of the sperm area. These fissures separate the layers previously mentioned. In position and outline this area corresponds very nearly to the 'gray crescent' of the frog's egg (Roux, '83, '85, '87 and '03; Schultze, '00; see also Jenkinson, '09, p. 80 and fig. 43).

Within eight to twelve hours after fertilization the sperm pits have become indistinct and, as a rule, they all disappear before the first cleavage, though cases have been found as late as the fifth cleavage stage. Meanwhile the sperm areas also become indistinct, losing the dark line which serves as a boundary and gradually blending with the surrounding surface of the egg. Fifteen or twenty hours after fertilization, it is usually impossible to orient the egg with respect to the point of entrance of a spermatozoon; before the egg is ready for first cleavage it has resumed the general appearance of radial symmetry which it had before fertilization.

The sperm areas have not been observed in living material, but the examination was made without the aid of a binocular microscope, an instrument which has proved of great value in the surface study of the fertilization stage with preserved material.

In preserved material a space sometimes appears between the blastodisc and the vitelline membrane which elsewhere closely invests the egg. An examination of living eggs at intervals from fertilization to first cleavage shows that normally the vitelline membrane fits closely about the entire egg. The condition noted in preserved material is due to the subsidence of the blastodisc; the vitelline membrane does not spring away from the egg after fertilization, as occurs in some lower forms.

If one remove an unfertilized egg from its gelatinous envelope and immerse it in water, and place almost in contact with it a drop of seminal fluid, one observes that the spermatozoa by means of slow writhing movements disperse gradually in all directions. There is no evidence of attraction by the egg, but spermatozoa coming in chance contact with it adhere to its surface, so that in

time there are more spermatozoa at the surface of the egg than at a little distance from it. As previously noted, spermatozoa are found in capsules that do not contain eggs; in this case there is no possibility of attraction by the egg.

In *Cryptobranchus*, as in other amphibian eggs, there is no preformed micropyle. In eggs fertilized in a natural manner, the spermatozoon may enter the egg at any point. More sperm pits have been found in the marginal region of the blastodisc, about midway between the equator and the animal pole, than elsewhere, indicating that this zone may be especially favorable to the entrance of the spermatozoon; but if any selective influence is at work, it cannot be a strong one, for spermatozoa have been found penetrating the egg close to the second polar spindle, and at various points in the lower hemisphere, even at the vegetal pole. Sperm areas are best developed about those sperm pits that occur near the margin of the blastodisc. In only one case has a sperm pit at the vegetal pole been found surrounded by a sperm area. Sperm pits are often more numerous on one side of the egg than on the opposite side, indicating a chance inequality in the exposure of the egg to the seminal fluid.

All the statements in this section regarding penetration of the egg by the spermatozoa have been confirmed by sectioning eggs which have first been carefully described externally.

D. SUMMARY

A germinal area is first visible in the ovarian egg taken about the middle of August. The germinal area is usually situated on the more exposed side of the egg, toward the periphery of the ovary; it has at first a diameter of about 60° , and increases gradually in size until about the time of first cleavage; it has then a diameter of about 145° .

In ovarian eggs examined about the first of September, the germinal vesicle is usually visible at the surface, in the center of the blastodisc; it disappears shortly before the egg leaves the ovary.

Soon after the eggs have reached the uterus, a sharply-defined pit appears at the animal pole; this pit persists up to the time of

first cleavage. About the time of the appearance of the pit at the animal pole, the egg becomes capable of fertilization.

The point of entrance of a spermatozoon (the 'sperm pit') is easily recognizable in both living and preserved eggs. In preserved material, the influence of the spermatozoon on the egg substance is indicated in surface views by the differentiation of a large circular area (the 'sperm area') surrounding the sperm pit. This area is recognizable by a slight difference in color and by the presence of a bounding dark line; it increases in size until it covers nearly a hemisphere of the egg, then disappears.

In artificially fertilized eggs, and presumably in eggs fertilized in nature, polyspermy usually occurs.

There is no evidence of attraction of the spermatozoon by the egg.

The spermatozoon may enter the egg at any point, but sperm areas are best developed about those sperm pits that occur near the margin of the blastodisc.

V. THE INTERNAL HISTORY OF THE EGG BEFORE CLEAVAGE

The present section deals with a few features concerned in ovogenesis and maturation, and gives a more detailed account of the fertilization phenomena.

A. OVOGENESIS

The material for this study consists as follows:

(a). For the early stages it was found best to use larval and immature post-larval females, with a body-length ranging from 9 to 38 cm. (two years old and upward). Females with a body length of more than 38 cm. are almost always sexually mature.

(b). The residual eggs of spent females taken in September furnished ovocytes slightly older than those of the largest immature females taken in August and September.

(c). Mature females taken during July and August furnished material for the late stages of ovogenesis.

There remains a period during the last year of development, extending from October to June inclusive, which is not represented

in the material. Since during this time, which includes the winter months, development is least active, the lack of these stages is of minor importance for the purposes of the present paper.

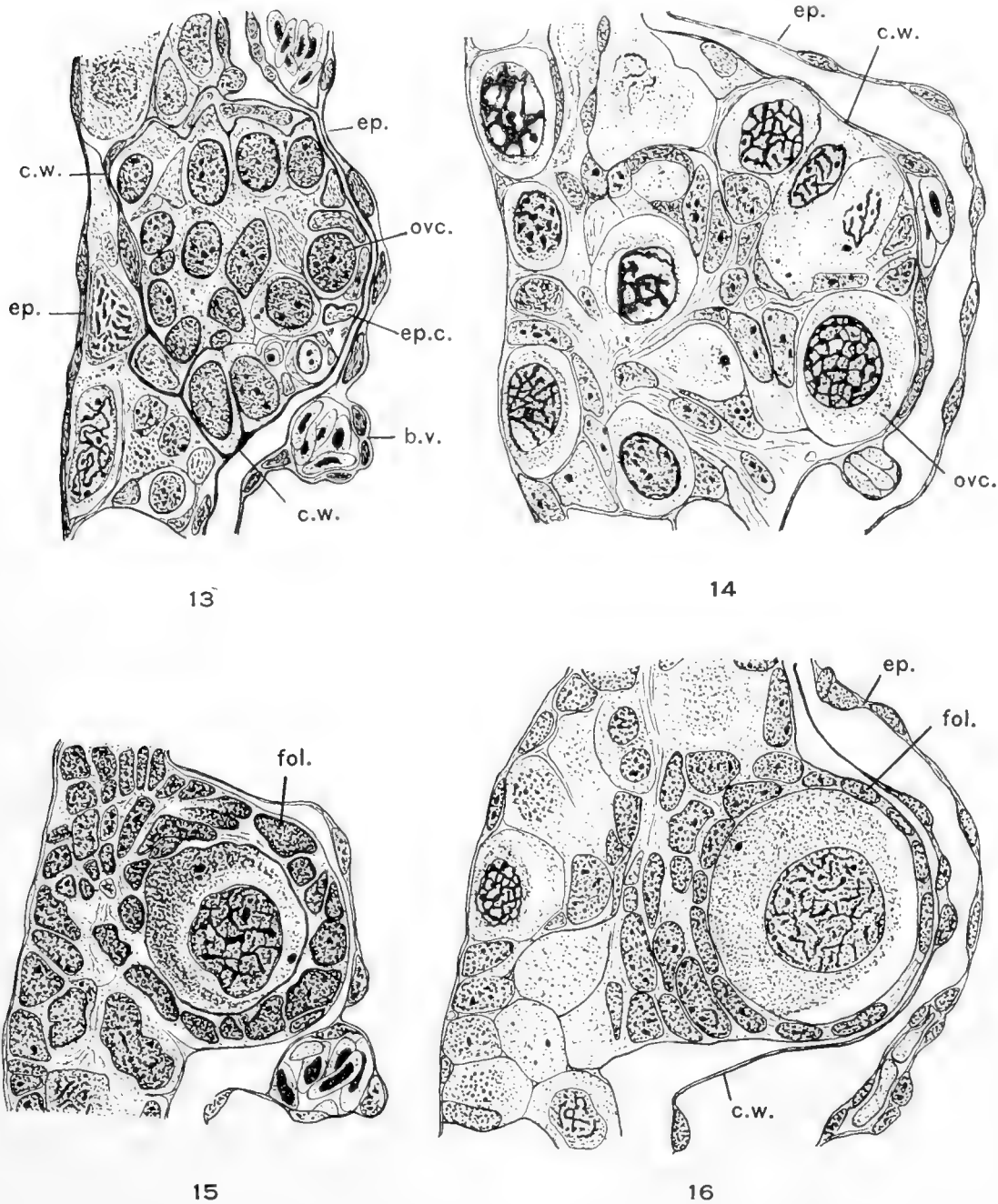
1. The formation of the follicle and the egg membranes

The young ovary of *Cryptobranchus* is essentially a sac with thick cellular walls. In a 9 cm. larva the ovarian wall (see figs. 13 to 17) shows structural differentiation as follows: (a) an inner and an outer limiting membrane of flattened epithelium; these membranes are connected by (b) a network of cells of a character similar to those comprising the limiting membranes, though usually not so greatly flattened; within the meshes of this network are found (c) young ovocytes in various stages of development.

In the ovary of a 9 cm. larva, more or less clearly defined groups or cysts of very young ovocytes (see fig. 13) may be found, each group surrounded by a thin epithelial membrane, the cyst membrane. All the ovocytes of each group or cyst are presumably the product of a single primary ovogonium. Epithelial cells also occur within the cyst. Within many of these cysts, development has gone further, and some or perhaps all the ovocytes have undergone an increase in size which involves both nucleus and cytoplasm (see fig. 14). Within each cyst, one ovocyte usually outstrips its fellows, and becomes surrounded by a layer of epithelial cells which form the follicle (fig. 15).

With a further increase in size of the ovocyte, the follicular layer assumes the character of a definite membrane with somewhat flattened cells, and that portion of the cyst membrane in contact with the ovarian membrane shows an increase in the number of its nuclei and is more clearly differentiated as a separate layer (see fig. 16).

With a still greater increase in size, as shown by the most advanced ovocytes of a 9 cm. larva and in later stages, the egg presses the overlying membranes into the central cavity of the ovary, so that the ovocyte comes to be suspended as in a sac, and is more nearly surrounded by the cyst and ovarian membranes (see figs. 17 to 21). In all three membranes, an increase in the



Figs. 13 to 16 Cross-sections through the wall of the ovary of a 9 cm. larva of *Cryptobranchus allegheniensis*. $\times 300$. *b. v.*, blood vessel; *c. w.*, cyst wall; *ep.* (right), inner epithelial membrane of the ovarian wall; *ep.* (left), outer epithelial membrane of the ovarian wall; *ep. c.*, epithelial cell of the cyst; *fol.*, follicle cell; *ovc.*, ovocyte.

Fig. 13 A cyst containing young ovocytes and epithelial cells occupies the central part of the figure.

Fig. 14 A cyst containing slightly older ovocytes.

Fig. 15 An ovocyte surrounded by the newly-formed follicle.

Fig. 16 An ovocyte and follicle slightly more advanced than the one shown in preceding figure.

number of nuclei keeps pace with the increase in extent. In a 35 cm. female (see figs. 21 and 22), the nuclei of the follicular membrane are the most numerous and least flattened; those of the cyst membrane and inner ovarian membrane are both decidedly flattened. Somewhat rarely, the cyst membrane is ruptured by the expansion of the ovocyte. According to King ('08) in *Bufo* the rupture of the cyst membrane takes place regularly at an early stage.

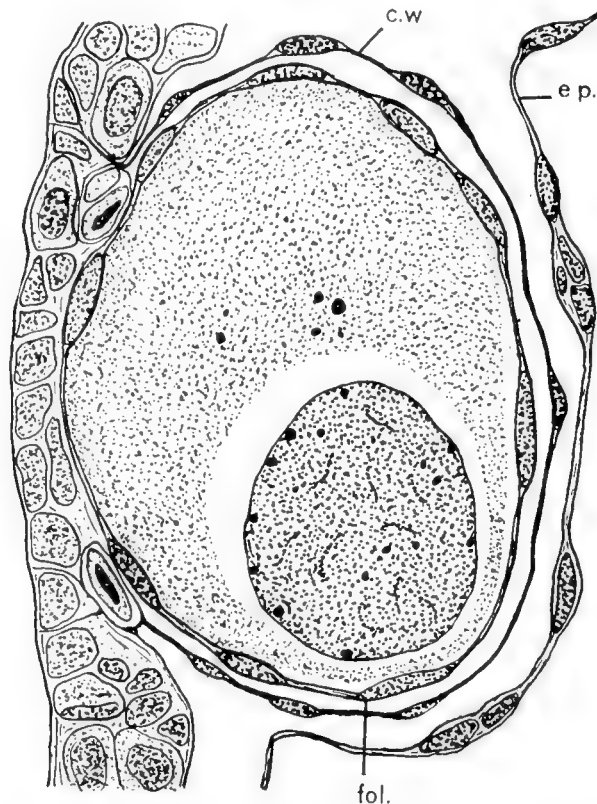


Fig. 17 Cross-section through the ovarian wall of a 9 cm. larva of *Cryptobranchus alleganiensis*, showing one of the most advanced ovocytes. $\times 300$. Lettering as in the preceding figures.

The ovocyte in the advanced growth stage is thus surrounded by a single-layered follicle, suspended in a flask-shaped two-layered sac of which the inner layer is the cyst membrane, the outer layer is the inner epithelial membrane of the ovarian wall. In a broader sense, the entire three-layered structure may be called a follicle, and the neck of the flask-shaped sac may be called the stalk of the follicle. This triple-layered wall persists without any radical change in structure up to the time of maturation.

In the later stages of the development of the ovary, its walls anastomose by the formation of cross-walls or partitions, dividing the ovary into compartments or perhaps pockets; by these cross-walls the course of the inner ovarian membrane is greatly complicated.

The ovocyte of a female of 26 cm. and younger is apparently a naked cell, possessing no proper membrane. In females with a

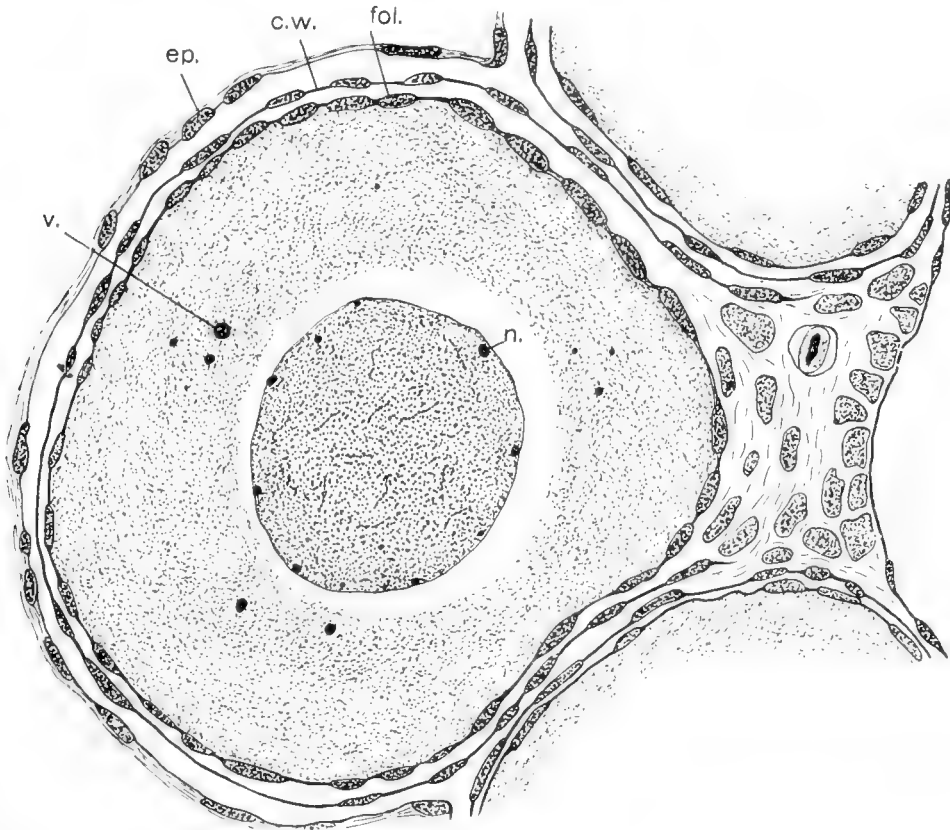


Fig. 18 Cross-section through the ovarian wall of a 26 cm. *Cryptobranchus allegheniensis*, showing one of the most advanced ovocytes. $\times 180$. *n.*, nucleolus; *v.*, vitelline body; *ep.*, inner epithelial membrane of the ovarian wall. Other lettering as in figs. 13 to 16.

body length of from 30 to 35 cm. there occurs a rapid development of two non-cellular membranes closely investing the egg within the follicle. The inner of these two membranes exhibits a radial striation and is the zona radiata; at the time of maturation it becomes a simple cell wall to the egg. The outer membrane, clear and homogeneous, is the zona pellucida; it persists as the 'vitelline membrane' of the embryo.

The zona radiata and the zona pellucida begin to form simultaneously, shortly before the appearance of yolk granules. In the most advanced ovocytes of a 35 cm. female, these membranes are well established and a narrow zone of yolk has appeared near the periphery of the ovocyte (see fig. 22).

The zona radiata arises from the peripheral cytoplasm of the ovocyte. In its early stages its inner boundary is not sharply defined; its staining reaction is like that of the egg cytoplasm; aside from its cross-striation its structure, like that of the egg cytoplasm, is finely granular. The zona pellucida, on the other hand, is formed *de novo* as a product of cellular activity. In the ovary of a 35 cm. female its staining reaction is different from that of any other structure present: with the borax-carminc Lyons-blue picric-acid mixture it becomes green, while the ground-substance of the follicular, cyst and ovarian membranes stains blue. Since, later, a membrane exactly resembling the zona pellucida in character sometimes, though not typically, forms between the cyst membrane and the follicle (see fig. 32), it seems reasonable to conclude that the zona pellucida is the product of the follicle rather than of the egg.

In the most advanced ovocytes of a spent female there is usually an increase in the thickness of the zona pellucida, while the zona radiata shows signs of degeneration—there is a slight loss in the distinctness of the radial striations. In adult females taken in July and August, there is a further loss in the distinctness of the striations of the zona radiata. In ovocytes taken just before maturation, with the germinal vesicle close to the surface, the zona radiata has in some cases almost lost its radial striation, is decreased in thickness, and is becoming a simple cell wall to the egg.

The literature on the zona pellucida and zona radiata of the amphibian egg has been reviewed by Waldeyer in Hertwig's ('06) *Handbuch* and needs no summary here.

The ovary of a young *Necturus* 20 cm. long, killed August 25, gives stages corresponding to those of a 35 cm. *Cryptobranchus*. The follicular layers and mode of attachment of the ovocyte to the ovarian wall are practically the same as in *Cryptobranchus*,

with the exception that there is a marked difference in the appearance of the nuclei of the follicle proper: in *Necturus* these nuclei are more numerous, and in form are spherical or even elongated in a radial direction, instead of being flattened in the direction of the circumference of the egg as in *Cryptobranchus*. The follicle of *Necturus* more closely resembles that of the selachian egg in an early stage (see Hertwig's *Handbuch*, '06, figs. 105 and 195). The zona pellucida and zona radiata are much alike in the two urodeles; the striations of the latter membrane are rather more distinct in *Necturus*.

2. The establishment of polarity, and the progress of axial differentiation

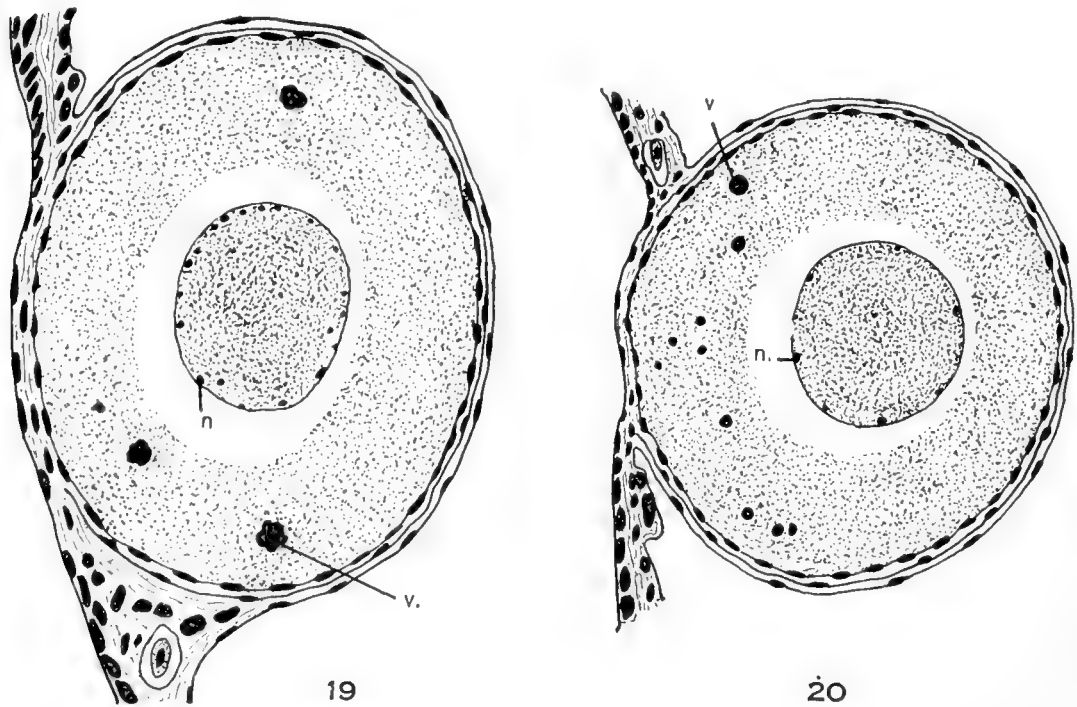
As already noted in the surface study of the ovarian egg, the ovocyte ready for maturation shows its telolecithal character in the presence of a superficial germinal area, in the center of which lies the germinal vesicle, while the remainder of the egg is heavily laden with yolk. It is the purpose of the present section to trace the changes by which this axial differentiation is brought about.

In the ovary of a 9 cm. larva, vitelline bodies (see King, '08) are recognizable in the cytoplasm of the ovocytes in all stages present, but are not very numerous nor conspicuous even in the most advanced ovocytes of such an ovary (see figs. 13 to 17). In the largest ovocytes, the germinal vesicle is usually somewhat excentrically situated, but with no constancy in the direction of excentricity. Faintly-staining nucleoli are distributed quite promiscuously throughout the germinal vesicle, in the later stages with a slight tendency toward forming a ring at the periphery.

In the most advanced ovocytes of a 26 cm. female (see fig. 18) there is less excentricity in the position of the germinal vesicle; the nucleoli are most numerous at the periphery. There is an increase in the number and size of the vitelline bodies, which are more numerous on the side toward the central cavity of the ovary. After fixation in Zenker's fluid, both nucleoli and vitelline bodies take the nuclear stain, though faintly. In the ovary of a 27 cm.

female, fixed in Bouin's solution, the nucleoli take the nuclear stain very faintly; the vitelline bodies take the cytoplasmic stain.

In the most advanced ovocytes of a 30 cm. female (figs. 19 and 20) the germinal vesicle is quite centrally situated—a position which it retains until a very late stage of ovogenesis. The nucleoli, which still stain but faintly, are nearly all at the periphery, where they form a uniform ring. The vitelline bodies shown in the figures now stain brilliantly with borax carmine used after



Figs. 19 and 20 Sections through ovocytes and ovarian wall of a 30 cm. *Cryptobranchus allegheniensis*, showing the follicle and the distribution of vitelline bodies and nucleoli. $\times 90$. *n.*, nucleolus; *v.*, vitelline bodies.

Zenker's fluid; in general they are much more numerous on the side toward the periphery of the ovary, in the region of the future animal pole. Some of the vitelline bodies are very large; these usually occupy an equatorial position, but are sometimes found on the inner side of the ovocyte. Comparison with the preceding stage suggests that the vitelline bodies originate on the inner side of the ovocyte and migrate to the outer side; that they reach their greatest development midway in the course of migration, and break up to form the smaller and more numerous vitelline bodies

in the region of the future animal pole. But scattered throughout the cytoplasm are occasionally to be found other bodies, resembling the vitelline bodies but more irregular in form and staining very faintly. While it is possible that these bodies are different in kind from the brilliantly-staining vitelline bodies, their appearance suggests that they are stages in the degeneration of the latter. The faintly-staining bodies, though seldom numerous, are more frequently found in regions poor in deeply-staining vitelline bodies. These observations enable us to offer an explanation of the distribution of vitelline bodies, alternative to the theory of migration: a wave of development of vitelline bodies, followed by a wave of degeneration, may sweep from the inner to the outer hemisphere of the ovocyte. But whether migration is real or only apparent, the fact remains that the region of most abundant deeply staining vitelline bodies has shifted from the vicinity of the future vegetal to the future animal pole of the ovocyte. This change is perhaps an expression of polarity; if so, it is the first indication of polarity that I have observed. However, it is not at all certain that polarity is not present at an earlier period; in particular the history of the chromatin has not been sufficiently studied, moreover it is of course possible that a physiological polarity of the cell may precede its manifestation in a visible form.

In the ovary of a 34 cm. female, fixed in Flemming's solution, the distribution of vitelline bodies is much the same as noted in the 30 cm. female; in form the vitelline bodies are sometimes oval or irregular, but never mulberry-shaped as is sometimes the case with Zenker's.

In the ovary of a 35 cm. female, yolk granules are beginning to form in the most advanced ovocytes; other ovocytes nearly as large contain no yolk. In neither of these two stages are vitelline bodies in the typical condition present, but they are sometimes found undergoing a process of degeneration—they lose the intensity of their staining reaction, become irregular in form, and disappear. The disappearance of the vitelline bodies at the time of the formation of yolk suggests a correlation between the two phenomena; but so far as I have been able to observe, the final stages

in the disappearance of the vitelline bodies are not closely associated with the formation of yolk granules, nor have I found any undoubted 'yolk nuclei,' such as have been described by King ('08) for *Bufo*. In view of the diversity in the methods of yolk-formation described for different amphibians, this result is not altogether surprising.

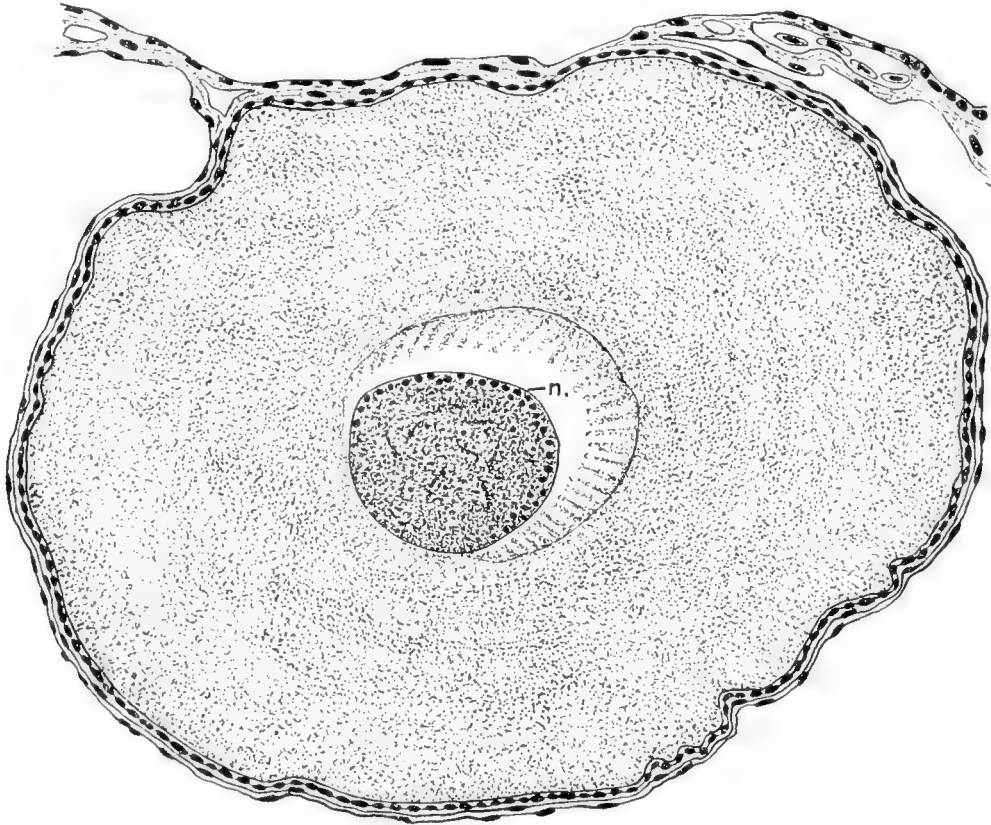


Fig. 21 Section through an ovocyte and ovarian wall of a 35 cm. *Cryptobranchus allegheniensis*, showing the follicle and the distribution of nucleoli. In this ovocyte the vitelline bodies have disappeared, but yolk-formation has not yet begun. $\times 60$. *n.*, nucleolus.

In the more advanced ovocytes of a 35 cm. female (see fig. 21). the nucleoli stain deeply with borax carmine used after Zenker's fluid. There is usually a marked concentration of the nucleoli on the side of the germinal vesicle toward the periphery of the ovary. Account must be taken of the fact that shrinkage of the germinal vesicle also proceeds, as a rule, most extensively on this side, leaving a large space, while the opposite side remains in contact with the cytoplasm. This greater shrinkage on the

outer side in part accounts for the greater concentration of nucleoli on this side, but it is inadequate to account for all of it; moreover the axis of excentricity in form due to shrinkage does not always correspond accurately to the axis of excentricity in the arrangement of the nucleoli.

This excentric distribution of material marks an axis which corresponds, roughly at least, to the polar axis at the time of maturation; the nucleoli accumulate on the side which is to become the animal pole, and thus perhaps afford a second indication of polarity. King ('08) found this condition in *Bufo* at the time when the nucleus was moving from the center of the egg to the animal pole, and suggested the possibility that the accumulation of most of the nucleoli in one part of the nucleus might have something to do with this movement. In *Cryptobranchus* this concentration of the nucleoli begins long before the migration of the germinal vesicle to the surface, and indeed before the formation of any yolk; it is most marked in the advanced ovocytes of a 35 cm. female, when the yolk is just beginning to form. As will appear from the study of later stages, this arrangement of the nucleoli does not persist during the actual migration of the germinal vesicle; nevertheless the early occurrence of axial concentration of nucleoli is significant.

In the ovary of a 35 cm. female, we find that occasionally, through the folding of the ovarian wall, an ovocyte has been thrust deep into the central cavity and has come in contact with the nutrient ovarian wall of the opposite side. The side opposite the stalk of the follicle now becomes the side best nourished, and here the nucleoli accumulate. Thus nature's experiment shows that the accumulation of nucleoli, and perhaps polarity, is not something predetermined in the egg, or even fixed by the relation of the egg to the ovarian wall within which it develops, but is a phenomenon depending upon larger environmental relations which probably have to do with nutrition; for as a consequence of the changed position of the egg the nucleoli accumulate on the opposite side from that favored by the original environment.

In the ovocytes of immature females with body lengths of from 35 to 38 cm., the yolk first appears in a narrow zone near the periph-

ery and parallel to the newly-formed zona radiata, but separated from the latter by a narrow layer of clear cytoplasm (see fig. 22). At this time the ovocyte has a diameter of from 1.5 mm. to 2 mm. The yolk zone is divisible into two layers, an outer

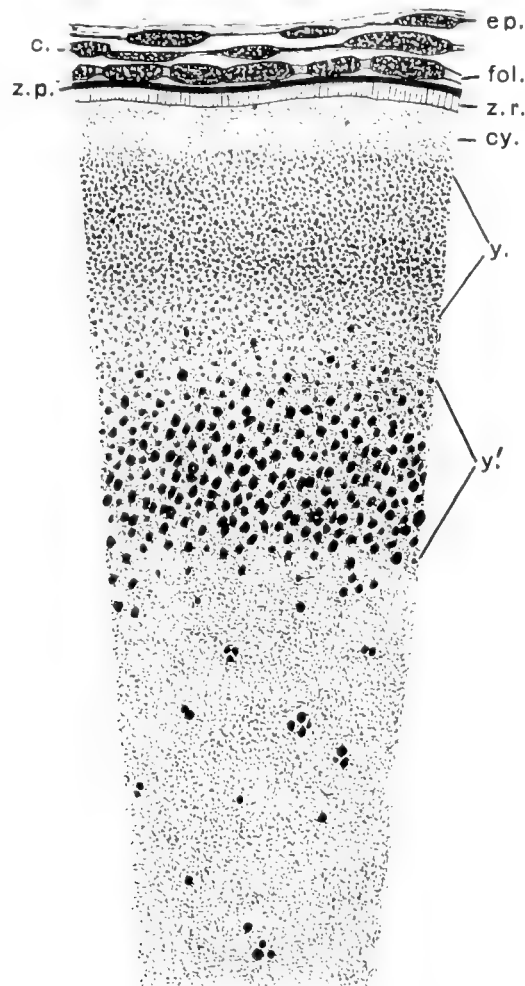


Fig. 22 Portion of a section through one of the most advanced ovocytes of a 35 cm. *Cryptobranchus alleghehiensis*, showing structure of the membranes surrounding the egg and the distribution of yolk granules. $\times 340$. The strip shown extends about half-way to the germinal vesicle. *c.*, cyst membrane; *cy.*, yolk-free peripheral zone of cytoplasm; *ep.*, inner epithelial membrane of the ovarian wall; *fol.*, follicular membrane proper; *z. p.*, zona pellucida; *z. r.*, zona radiata; *y.* and *y'*, layers of fine and coarse yolk granules respectively.

layer of fine yolk particles and an inner layer of coarse yolk particles, separated by a narrow region poor in yolk.

In the largest residual eggs (2 to 3 mm. in diameter) of spent females, the yolk-laden zone has extended inward further than

outward; a very narrow zone of clear cytoplasm persists at the periphery, and a much broader zone containing only a few scattering yolk granules surrounds the germinal vesicle. The middle portion of the yolk zone is now filled with coarse yolk granules; its margins consist of fine yolk particles. The germinal vesicle is still centrally situated, and the arrangement of yolk zones and

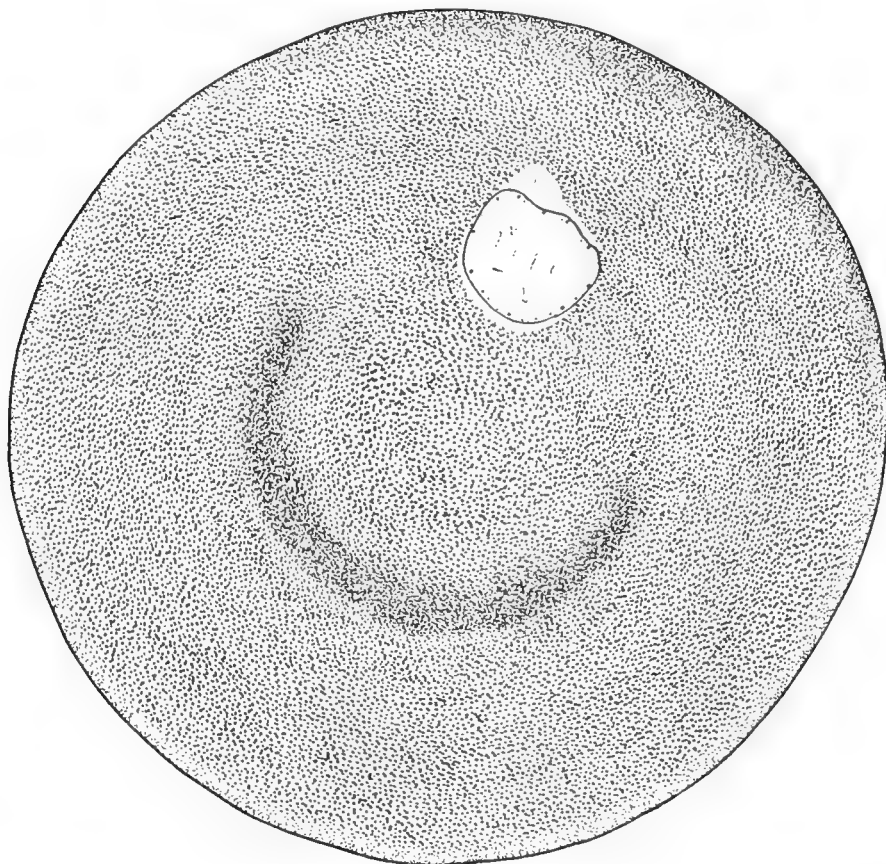


Fig. 23 Meridional section through one of the most advanced ovocytes of an adult *Cryptobranchus allegheniensis* killed July 6. The bounding line represents the zona radiata. $\times 20$.

cytoplasm is concentric. The nucleoli are still distributed at the periphery of the germinal vesicle, with only a slight tendency toward concentration at the outer side.

In the largest ovocytes of adults killed July 6 (see fig. 23), the germinal vesicle occupies a position midway between the center of the egg and the periphery, on the more exposed side of the egg, toward the stalk of the follicle. The animal pole is thus

defined by a point on the surface, toward which the germinal vesicle is moving. The cytoplasm is now everywhere thickly interspersed with yolk granules; these granules are in general coarse throughout the central portion of the egg, finer and more densely packed at the periphery. Axial differentiation in the arrangement of yolk particles is now for the first time evident in a slight thickening of the peripheral layer of fine yolk particles in the region of the animal pole. This region is also somewhat richer in cytoplasm than the remainder of the egg. There is thus present the beginning of a germinal disc or blastodisc, which in later stages becomes visible in surface views as the germinal area.

In the vegetal hemisphere a region of particularly fine and dense yolk, crescent-shaped in meridional section, lies mid-way between the center of the egg and the periphery. This region I shall call the 'yolk cup.' Its appearance suggests that it may be a part of a once continuous zone completely enclosing the germinal vesicle, and that, in the animal hemisphere, this zone has been interrupted in consequence of the migration of the germinal vesicle toward the surface. Probably the yolk cup is the physiological equivalent of the concentric layers of dense fine yolk found in the egg of the hen and various other vertebrates. Riddle ('11) has shown that the alternate layers of yellow and white yolk in the hen's egg are due to a daily rhythm in nutrition; he has advanced the same principle in explanation of the concentric layers of yolk in the eggs of certain cyclostomes, selachians and reptiles. In *Cryptobranchus*, from comparison with ovarian eggs taken in the autumn after the close of the spawning season, it is evident that in the stage under consideration the yolk cup marks the limits of growth during the preceding winter; hence it seems very probable that the yolk cup is the result of a seasonal variation in nutrition, and represents a layer added during the winter months.

The nucleoli are still found mainly at the periphery of the germinal vesicle, but with no constant tendency toward concentration in an axial position.

It has been noted that the animal pole, as defined both by the center of the germinal disc and the point on the surface toward

which the germinal vesicle is moving, lies in general on the more exposed side of the egg, within the stalk of the follicle. The animal pole thus lies in the opposite direction from that assumed in the ovarian egg of the hen (Lillie, '08, p. 29). According to King ('02) in the great majority of cases the egg of *Bufo* is attached in the equatorial region by the stalk of the follicle.

From a comparison of this stage with the preceding one (the ovocytes of a spent female), it is evident that yolk-formation proceeds concentrically about a centrally situated germinal vesicle until the egg is nearly or quite filled with yolk, and that axial differentiation in the arrangement of yolk particles does not appear until a very late stage of ovogenesis, two or three months before maturation. It is further apparent that the germinal vesicle attains its final position, not through unequal growth of the cytoplasm or excessive accumulation of yolk on the other side of the egg, but by a process of migration.

In the ovocytes of adults taken July 20, the germinal vesicle has migrated further toward the animal pole; it lies about one-third of the distance from the surface to the center of the egg. Both nucleoli and chromosomes are now aggregated at the center of the germinal vesicle. The yolk-cup persists, and there is an increase in the extent of the germinal disc. In some eggs a small cone-shaped mass of dense cytoplasm, with the apex of the cone pointing inward, lies immediately beneath the germinal vesicle.

In the ovary of an adult female killed August 17, the egg (fig. 24) has nearly reached its maximum size before fertilization; a meridional section cut in paraffin has a diameter of about 6 mm. (It should be noted that a yolk-laden egg does not shrink in paraffin to the same extent as ordinary tissues). The germinal vesicle lies only a short distance from the surface, and is bounded on the side toward the center of the egg by a large cone-shaped mass of cytoplasm. The apex of this cone is continuous with a slender meshwork of less dense but yolk-free cytoplasm extending half-way to the center of the egg. Owing to a slight obliquity of the slender cytoplasmic mass, it has not been found complete in any one section; in fig. 24 it has been added, from adjacent sections, to the one chosen for the remainder of the drawing.

Immediately beneath the zona radiata lies a peripheral layer of yolk-free cytoplasm, which from analogy with the teleost egg I shall call the 'protoplasmic mantle.' In the region of the vegetal pole this is so thin as to be barely recognizable with a magnification of 500 diameters; in the region of the animal pole it is thickened to form a disc which I shall call the 'cytodisc.' At the ani-

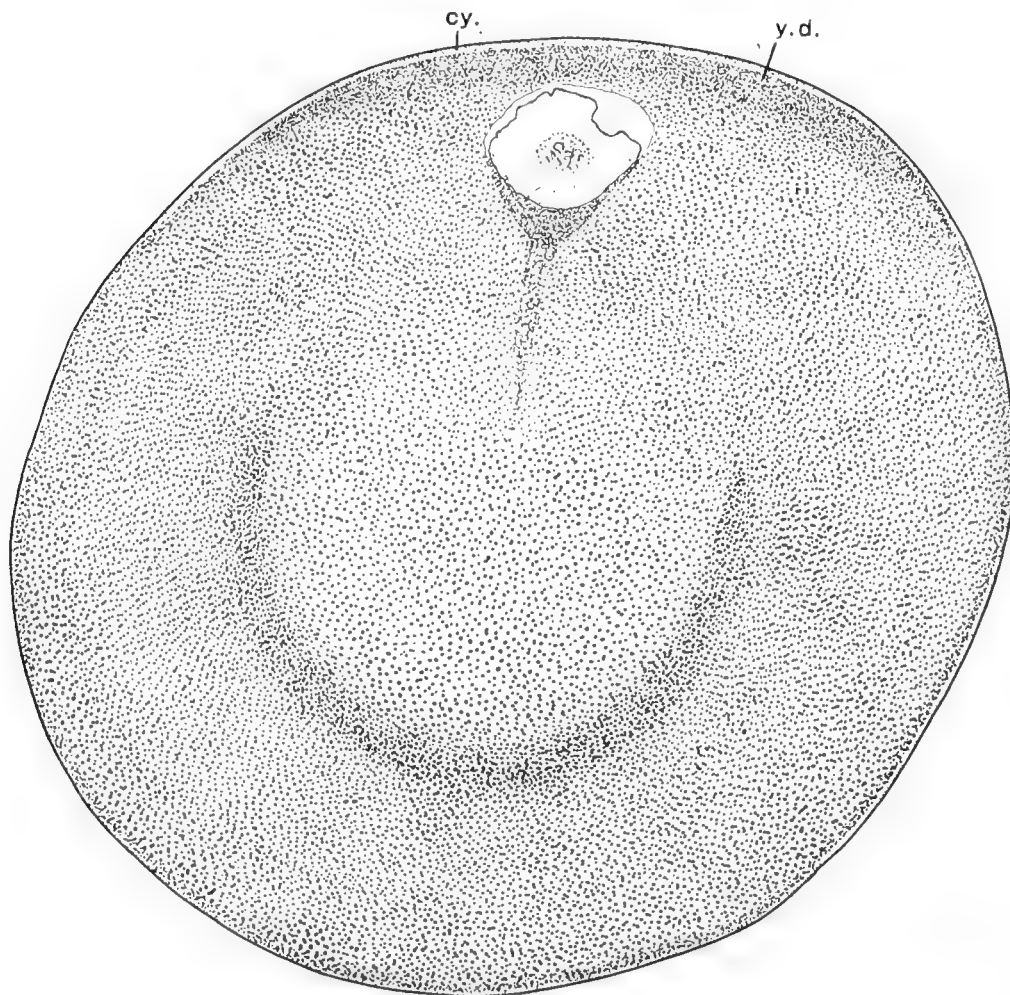


Fig. 24 Meridional section through an ovarian egg of an adult *Cryptobranchus alleggheniensis* killed Aug. 17. $\times 20$. *cy.*, cytodisc; *y. d.*, yolk disc.

mal pole the cytodisc reaches its maximum thickness of about 15μ —a little thicker than the layer of follicle cells proper.

The remainder of the egg is filled with yolk. Underlying the cytodisc and occupying an area about 100° in diameter surrounding the animal pole, is a thick layer of fine but dense yolk which I shall call the 'yolk disc.' The cytodisc and yolk disc combined

represent the anlage of the germinal disc or blastodisc, which later comes to enclose the germinal vesicle and the cytoplasm accumulated beneath it. Elsewhere a very thin peripheral layer of fine yolk particles, continuous with the yolk disc, lies immediately beneath the protoplasmic mantle. The interior of the egg shows no particular change in the yolk.

The germinal vesicle is spherical when perfectly preserved; when flattened this is due to shrinkage. The ground-substance or nuclear sap appears homogeneous under a low power, but with

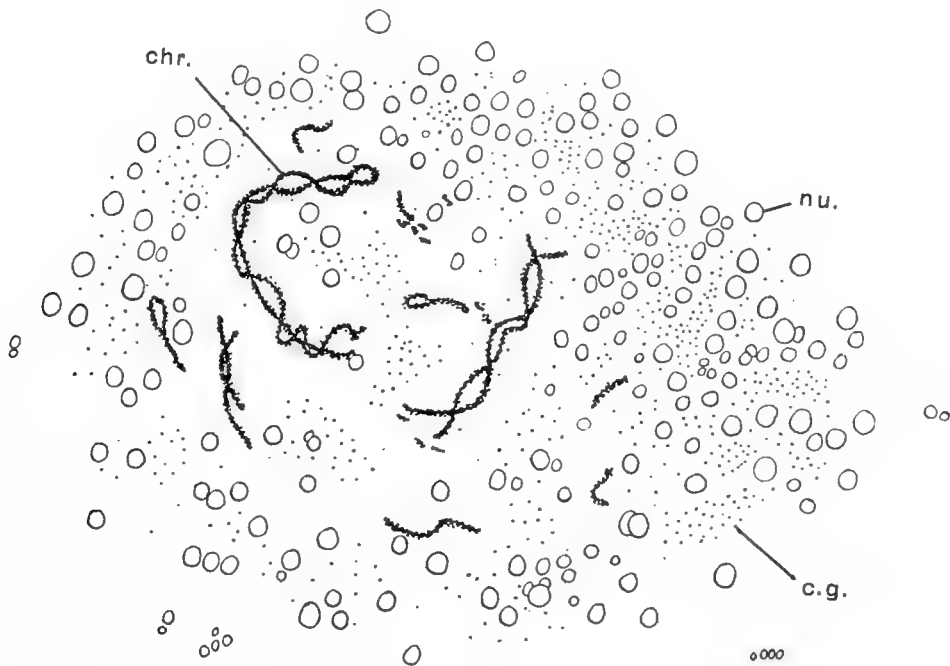


Fig. 25 Central portion of the germinal vesicle represented in the preceding figure, enlarged to show details. The finely granular ground-substance of the germinal vesicle is not shown. $\times 340$. *c. g.*, chromatin granules; *chr.*, chromosomes; *nu.*, nucleolus.

a magnification of 500 diameters it exhibits an extremely fine but dense granular structure. The nucleoli are now nearly all aggregated at the center; some few persist at the periphery, particularly on the side toward the center of the egg. The chromosomes are for the most part confined to the central part of the area occupied by the nucleoli.

Among the nucleoli, though not closely associated with them, there are now found very numerous and minute granules which

stain like chromatin (see fig. 25). These are evidently formed in close association with the chromosomes; in earlier stages chromosomes have been found covered with these granules before the latter have appeared elsewhere.

In the ovarian eggs of an adult killed August 22, the most marked changes in the general topography as viewed in meridional sections are a slight advance in the migration of the germinal vesicle toward the surface, and an increased thickness of the peripheral zone of fine yolk particles, particularly in the yolk disc. In the vegetal hemisphere the protoplasmic mantle is no longer recognizable as a separate layer; its constituents have mingled with the peripheral layer of fine yolk particles. The cytodisc is reduced in thickness by the blending of its inner surface with the yolk disc.

The narrow path of cytoplasm leading toward the center of the egg from the apex of the cone of cytoplasm underlying the germinal vesicle has disappeared; likewise the yolk cup is, as a rule, no longer present. In this stage there is a slight increase in the number of chromatin granules dispersed amongst the nucleoli; otherwise the nuclear contents seem unchanged.

Ovaries taken during the last week in August and the first week in September usually contain some eggs with the germinal vesicle appearing at the surface. In the general organization of the egg before the germinal vesicle actually reaches the surface, there are few changes from the condition described for August 22. Fig. 26 shows the general topography of an egg with the germinal vesicle very close to the surface. The cone of cytoplasm underlying the germinal vesicle is beginning to mingle with the yolk; it is not present in the section figured. Within the germinal vesicle the nucleoli are massed more closely together at the center; there is an increase in the number of chromatin granules, and apparently a gradual disappearance of the chromosomes—in some eggs they could not be found.

At the close of the period considered, axial differentiation is evident in the following arrangement of material: (*a*) the excentric position of the germinal vesicle and the cone-shaped mass of cytoplasm underlying it; and (*b*) the formation about the animal

pole of a germinal disc or blastodisc consisting of two layers, a very thin peripheral layer of yolk-free cytoplasm which has been called the cytodisc, and underlying this a thick lenticular layer of mingled cytoplasm and dense fine yolk which has been called the yolk disc. At any given level the egg is radially symmetrical about the axis of polarity. In general the egg has progressed from an alecithal through an isolecithal to a telolecithal stage.

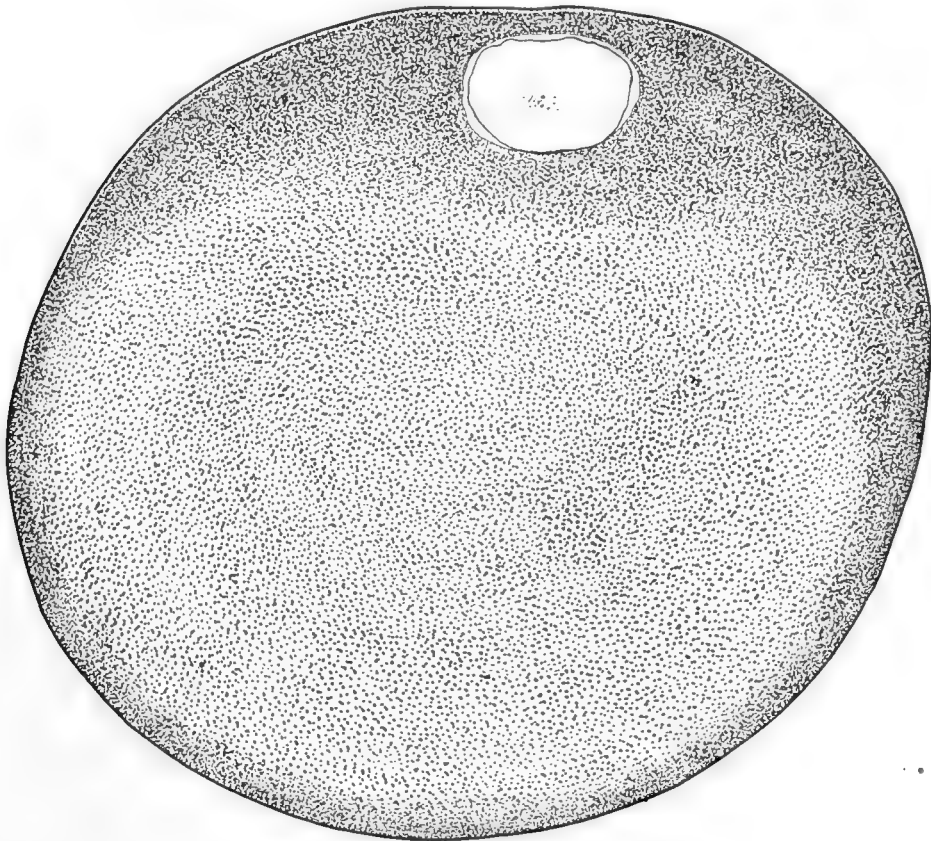


Fig. 26 Meridional section through an ovarian egg of an adult *Cryptobranchus allegheniensis* killed Sept. 6, 1910, showing organization just before the germinal vesicle reaches the surface. $\times 20$.

The establishment of polarity, with axial differentiation, is an event of great morphogenetic importance, since the formative materials for the embryo are being segregated in the vicinity of the animal pole. Through later changes in the distribution of this material the animal pole comes to mark the anterior, the vegetal pole the posterior end of the future animal; hence the establishment of polarity defines the principal axis of the embryo.

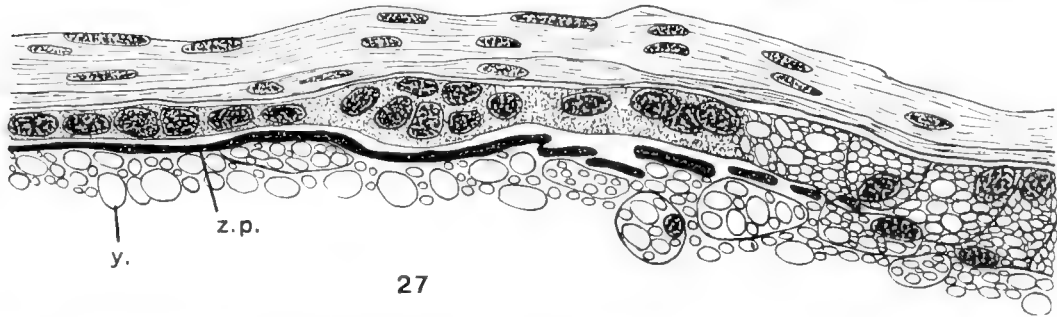
The changes that immediately follow—the appearance of the germinal vesicle at the surface, the rupture of its membrane, and the reorganization of the germinal disc with the incorporation of materials brought from the interior of the egg by the nucleus—lead up to maturation and will be considered in the account of that process.

3. Resorption of ovocytes; the follicle cells in a phagocytic rôle.

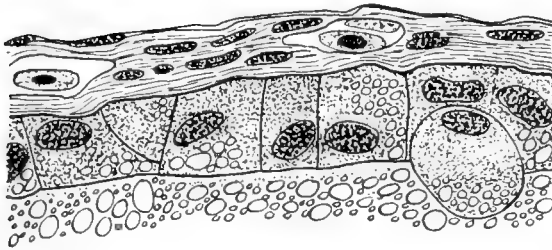
In young females nearing maturity (about 38 cm. body length), a few ovocytes reach an advanced stage of development, becoming filled with yolk and attaining a size nearly as great as the ovocytes of an adult. These precocious ovocytes fail to undergo maturation changes, and during the breeding season begin to degenerate, or rather to be resorbed, together with some of the less advanced ovocytes only partially filled with yolk. Viewed in the living ovary, these degenerating ovocytes are colored a very bright yellow or orange. Digestion and absorption of the yolk granules is accomplished through the medium of the cells of the follicular layer proper, which become greatly enlarged and function as phagocytes, thereby reversing their usual rôle as nurse cells to the egg.

The first step in the process of degeneration of the ovocyte is the disappearance of the zona radiata; the later stages are illustrated by figs. 27 to 30. The follicle cells enlarge, by increase both in the size of the nucleus and in the amount of cytoplasm. The zona pellucida is ruptured; at the same time it becomes irregularly thickened, a circumstance which may be interpreted either as a shortening of the fragments due to the release of tension, or as a step in the process of dissolution. The rupture of the zona pellucida allows the yolk to come in contact with the follicle cells; the latter engulf the yolk particles, and become surrounded by thin walls. About this time the zona pellucida disappears, and the follicle cells are left as large yolk-filled cells resembling columnar epithelium, forming a continuous layer around the egg.

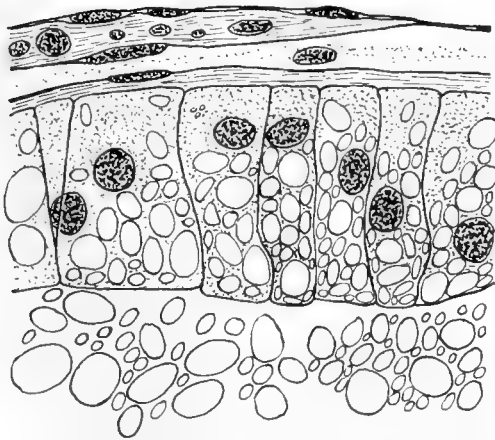
Digestion of the yolk particles is completed first at the outer margin of the follicle cells, while the inner margin continues to engulf yolk. The included yolk granules stain less deeply with



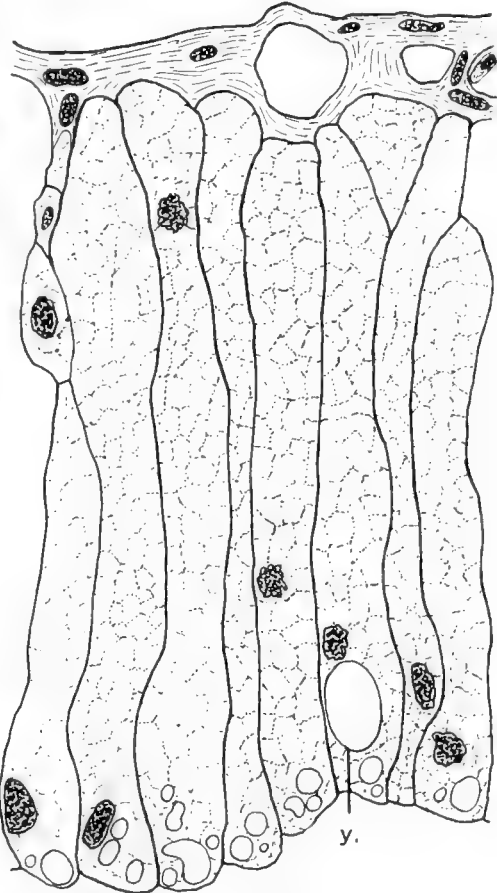
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Figs. 27-30 Changes at the periphery of an ovocyte in the process of resorption; the follicle cells are shown in a phagocytic rôle. Fig. 27, read from left to right, shows the beginning of the process (compare with fig. 22 showing the normal condition of the follicle). The remaining figures, taken from different ovocytes, show successively later stages. $\times 244$. *y.*, yolk; *z. p.*, zona pellucida.

haemotoxylin than the others, and in sections stained with the borax-carminé Lyons-blue picric-acid mixture the included granules take the cytoplasmic stain more deeply.

The follicle cells later become greatly elongated, and the cytoplasm takes the form of a faint meshwork with large spaces. Ingestion of yolk continues at the inner ends of the cells, while the remainder of the cell functions as a long tube to convey the products of digestion to the periphery. The follicular layer remains one cell in thickness until the cells have reached a length of about 250 μ ; with a further increase in thickness it becomes broken up into a meshwork of cells, amongst which are numerous capillaries. Ovocytes have been found in which this meshwork of cells reaches nearly to the center, and the remaining yolk is very small in amount.

In the adult female occasional eggs, though of full size, fail to escape from the ovary. Judging from their external appearance these ovocytes undergo resorption in the manner just described. A somewhat similar process of resorption has been described in the eggs of cyclostomes and fishes (Bühler, '02).

4. The organization of the egg shortly before the appearance of the germinal vesicle at the surface

At this point it may be well to summarize briefly the condition of the ovocyte in the stage immediately preceding the appearance of the germinal vesicle at the surface (see fig. 26).

The egg lies within a triple-walled follicular sac whose cellular membranes have undergone little change since they first became well established. The stalk of the follicle, and in general the animal pole of the egg, lie toward the periphery of the ovary.

The zona pellucida persists unchanged, except for a slight increase in thickness; the zona radiata shows signs of atrophy, and in some cases is assuming the character of a simple cell wall.

The nucleus or germinal vesicle has migrated from the center of the egg to a position near the periphery, ordinarily on the side toward the stalk of the follicle. During the migration of the germinal vesicle a cone-shaped mass of dense cytoplasm has

collected beneath it, and is now beginning to mingle with the surrounding yolk.

A germinal disc or blastodisc is evident in surface views of living material as a circular area, lighter in color than the rest of the egg, about 60° in diameter and situated on the more exposed side of the egg. In meridional sections it is shown to consist of two layers: a thin peripheral layer of cytoplasm, the cytodisc; underlying this a thick lenticular mass of mingled fine yolk particles and cytoplasm, the yolk disc. The germinal vesicle lies at the center of the yolk disc.

The yolk disc is continuous with a thin peripheral layer of fine yolk granules, mixed with cytoplasm, which lies in contact with the zona radiata everywhere except in the region of the cytodisc.

The remainder of the egg is filled with coarse yolk granules mingled with fine yolk granules and a small amount of cytoplasm.

The nucleoli are grouped at the center of the germinal vesicle, and amongst them are numerous chromatin granules. In some eggs chromosomes are found at the center of the group of nucleoli, in others the chromosomes have disappeared.

A point on the surface overlying the center of the germinal vesicle marks the animal pole. The general arrangement of materials is radially symmetrical about the axis of polarity, with differentiation proceeding in the direction of this axis.

B. MATURATION

1. The germinal vesicle at the surface

Meridional sections through ovocytes with the germinal vesicle at the surface show little change in the details of structure from the condition previously described. The germinal vesicle is usually somewhat flattened against the periphery, and a portion of its surface is in direct contact with the zona radiata. Masses of a wavy fibrous material are occasionally found in the nuclear sap. A few fragments of chromosomes are present in some eggs; in others no chromosomes have been found. The nucleoli and chromatin granules persist at the center of the germinal vesicle.

2. *The dissolution of the germinal vesicle, and the formation of the first polar spindle*

Material for the study of this stage was obtained from two females in which the majority of the ripening eggs had left the ovary, and were found distributed in the body cavity, oviduct and uterus. The nearly mature eggs left in these ovaries were found in every case investigated (nine eggs were sectioned) to have the germinal vesicle ruptured and its constituents well mixed with those of the blastodisc; in the majority of cases the first polar spindle had already formed.

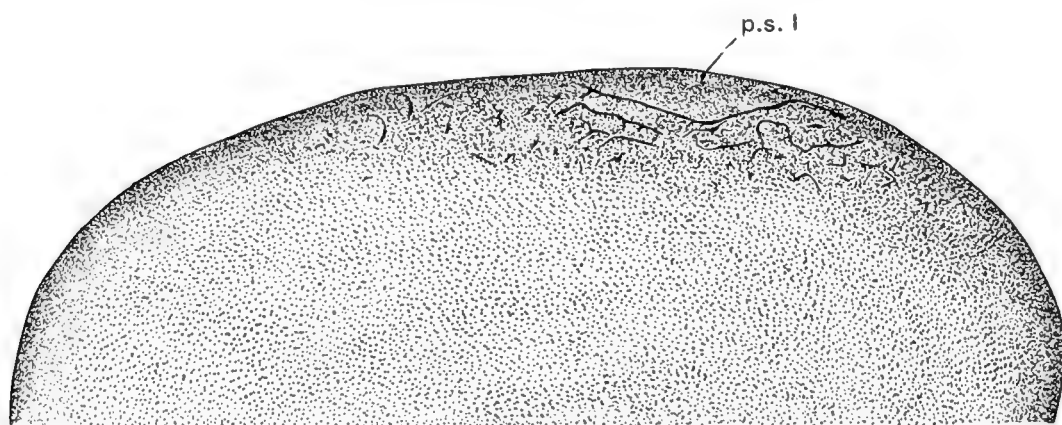


Fig. 31 Meridional section through an ovarian egg of *Cryptobranchus allegheniensis*, shortly after the rupture of the germinal vesicle. Fragments of the germinal vesicle are seen scattered throughout the blastodisc. $\times 18$. *p. s. I*, first polar spindle.

The rupture of the germinal vesicle and the distribution of its materials throughout the blastodisc must take place with considerable rapidity, since in eggs sectioned only the beginning and the end of the process have been observed. Fragments of the nuclear membrane, together with the wavy fibrous material previously noted in the germinal vesicle and innumerable fine granules, probably derived from the cell sap, become widely scattered throughout the germinal disc (see fig. 31). During this process of disintegration of the germinal vesicle the nucleoli and chromatin granules are lost to view. It seems improbable that all the chromatin granules should again be segregated as nuclear material; at any rate the rupture of the germinal vesicle affords an oppor-

tunity for the contribution of important nuclear material to the cytoplasm.

The germinal disc or blastodisc no longer shows a division into two layers; the material of the cytodisc is intimately mingled with that of the yolk disc. The cone of cytoplasm following the germinal vesicle in its migration is likewise more or less thoroughly incorporated into the blastodisc.

The end result of the migration of the germinal vesicle to the surface and its disintegration in that situation is now apparent. All the material of the nucleus and a considerable amount of cytoplasm have been brought from the interior of the egg to the vicinity of the animal pole, fragmented, and the *débris* more or less scattered throughout the blastodisc. Out of this complex there soon emerges close to the surface at the animal pole the reconstructed nucleus in the form of the first polar spindle. One function of migration is doubtless to get this nuclear material to the periphery where a part of it may be disposed of in the maturation divisions. A further adaptation is found in the fact that, later, the egg-nucleus or female pronucleus is left in the center of the formative material of the blastodisc. A third end attained by migration is that the formative material of the blastodisc is added to by cytoplasm following the germinal vesicle, and also by substances derived from the germinal vesicle itself.

The zona radiata has become reduced in thickness, has lost its striation and no longer shows a distinct limiting inner surface—its inner margin is irregular or blends with the peripheral cytoplasm of the egg. When the egg is shrunken away from the zona pellucida the zona radiata usually remains organically connected with the egg. The character of the zona radiata has changed so radically that it will no longer be referred to by this name; it has become a simple cell wall to the egg, and as such takes part in the later process of cleavage.

The zona pellucida persists unchanged as the so-called vitelline membrane of the egg at the time of fertilization and during the early stages of embryonic development.

As in other amphibian eggs, only these two membranes, the zona pellucida and the cell wall formed from the zona radiata,

accompany the egg in its escape from the ovary; the process of ovulation involves the rupture of the follicle which remains in the ovary.

The occurrence of the first polar spindle was studied in two females, (*A*) and (*B*), in which the eggs were distributed from ovary to uterus inclusive. The first polar spindle was found in eggs taken from the following situations: ovary, body cavity, oviduct, and extreme upper part of the uterus; out of a total of twenty-eight eggs studied, the first polar spindle was found in thirteen cases. Five eggs taken from the lower uterus were studied; no first polar spindle was found.

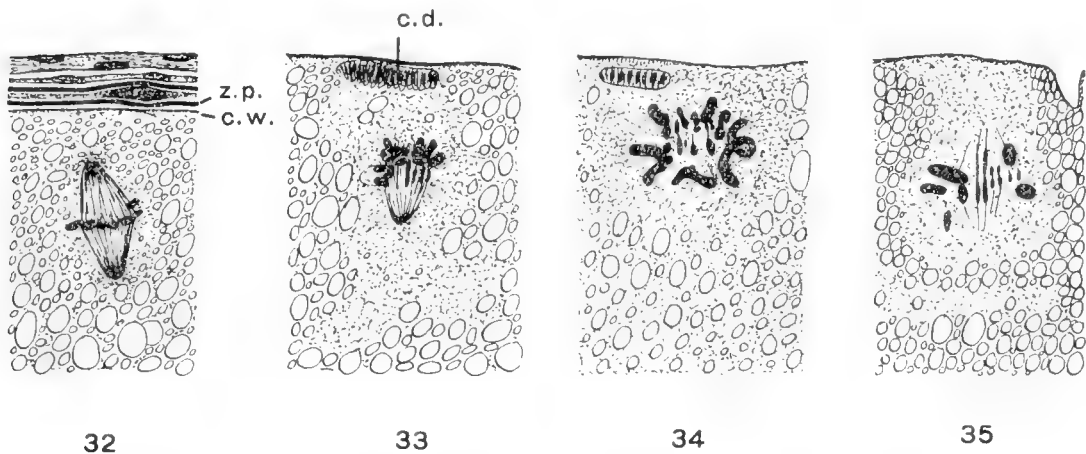
In the case of another female (*C*), in which the eggs were all in the uteri, no first polar spindle was found in three eggs sectioned.

Allowance must be made for the fact that in some cases in which the first polar body is absent it may have been missed on account of imperfections in the series. The results are sufficient to justify the conclusion that the first polar spindle is usually present at the time the egg leaves the ovary and during its passage down the oviduct, and that it disappears about the time the egg reaches the uterus.

The first polar spindle (see figs. 32 to 35) is formed with its long axis either coinciding with the axis of polarity of the egg, or oblique to this axis. The number of chromosomes is probably twelve before any of them have divided. There is an outer ring of six large chromosomes, surrounding a central group of six small chromosomes usually found in a state of division; it is probable that these six small chromosomes are not all of equal size. These size differences of the chromosomes are interesting in the light of well-known recent work indicating individual differences in the chromosomes of many forms.

There is frequently present close to the cell wall overlying the spindle a disc-shaped body with an irregular cross-striated structure, which, from its probable mode of origin, I shall call the 'contact disc' (see figs. 33 and 34). This disc takes the cytoplasmic stain, and seems to be of the same composition as the cell wall. The adjacent cell wall is slightly thickened and sometimes shows a cross-striation, reminding one of the zona radiata (compare the

effect on the cell wall of penetration by the spermatozoon, described later). The presence of the contact disc is uniformly accompanied by a deficiency of the spindle, which lacks an aster at the end nearest the disc. In a few cases there seems to be a small amount of sphere substance underlying the contact disc. The inference seems to be that the contact disc is the product of the aster of the first polar spindle modified by contact with the



Figs. 32-35 Meridianal sections showing first polar spindle of *Cryptobranchus allegheniensis*. Figs. 32 to 34 are from ovarian eggs; fig. 35 is from an egg taken from the lower part of the oviduct. $\times 340$.

Fig. 32 *c. w.*, cell wall, formed from the zona radiata. *z. p.*, zona pellucida.

Fig. 33 *c. d.*, contact disc.

Fig. 34 The section cuts the spindle obliquely and includes all the chromatin except one small chromosome belonging to the central part of the group, which is left in an adjacent section. There are probably six large chromosomes forming a ring, surrounding six small chromosomes in a state of division.

Fig. 35 A considerable part of the chromatin is left in an adjacent section. There are probably six large and six small chromosomes, arranged much as in the preceding figure.

cell wall. The function of the disc, if it have any function, may be to anchor the spindle at the surface during the pulling-apart of the two sets of chromosomes. Unfortunately for this hypothesis the linin threads have not been traced from the chromosomes of the first polar spindle to the contact disc; but since the latter structure is never found except in conjunction with a polar spindle, there is no escape from the conclusion that it is in some way related to it.

Sections afford no explanation of the faint dark spot or shallow depression noted in surface views of the animal pole after the rupture of the germinal vesicle and before the formation of the second polar spindle. An actual depression overlying the first polar spindle is rarely found in sections; if present in the living egg it must be lost through shrinkage of the egg during the process of preparation for sectioning by the paraffin method.

The yolk granules immediately adjacent to the cytoplasm surrounding the spindle are distinctly larger than at the same level elsewhere; they are doubtless brought from a deeper situation by the migration of the nucleus.

The anaphase of the first polar spindle has not been observed, and the first polar body has been found only in a state of degeneration, in conjunction with the second polar spindle.

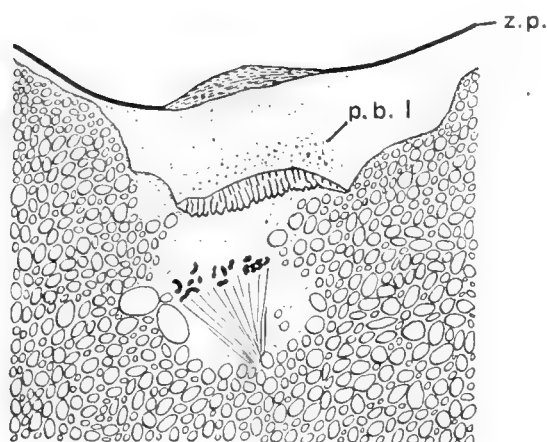
3. The second polar spindle

The second polar spindle (see figs. 36–38) may be distinguished from the first by the smaller amount of chromatin material, and by the fact that a well-defined pit already noted in surface views usually lies above it. This pit sometimes disappears in the late anaphase of the spindle.

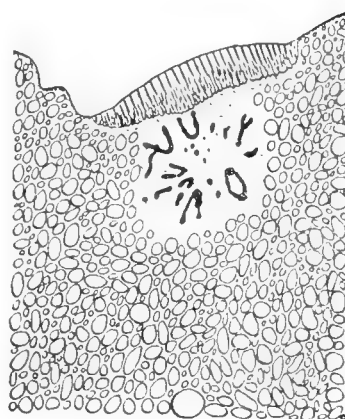
The débris of the first polar body is usually found at the bottom or sides of the pit, outside of the cell wall; in some cases fragments of its chromatin are found mingled with the contact disc of the first polar spindle. The chromatin fragments stain but faintly with borax carmine.

The contact disc of the first polar spindle has fused with the thickening of the cuticle which overlies it. In the telophase of the second polar spindle a new contact disc is formed which soon fuses with the old. In some cases linin threads have been clearly traced from chromosomes to the contact disc of the early second cleavage spindle, thereby sustaining the view of the origin of the contact disc set forth in the account of the first cleavage spindle.

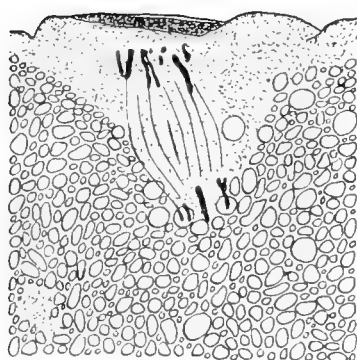
No second polar spindle has been found in any eggs of the two females, (A) and (B), for which the occurrence of the first polar spindle was tabulated. This indicates that the second polar spindle is not formed until after the eggs have been for some time



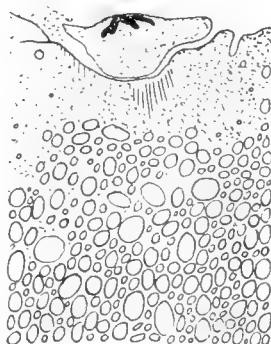
36



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Figs. 36 to 40 Meridional sections showing the second polar spindle, and the formation of the second polar body and the egg-nucleus in *Cryptobranchus allegheniensis*. $\times 340$.

Fig. 36 Section through the second polar spindle of an unfertilized egg taken from the uterus of a ripe female. *z. p.*, zona pellucida; *p. b. I*, debris of the degenerating first polar body.

Fig. 37 Section through the second polar spindle of an egg killed 15 minutes after fertilization. The section lies in the plane of the equator of the spindle.

Fig. 38 Late anaphase of the second polar spindle in an egg killed $2\frac{1}{2}$ hours after fertilization. A considerable part of the chromatin is left in an adjacent section.

Figs. 39 and 40 Two consecutive sections through an egg killed 5 hours after fertilization; the first figure shows the second polar body, the second figure shows in addition the newly-formed egg-nucleus.

in the uterus. In the third female (*C*) considered, in which all the eggs were in the uterus, a second polar spindle was found in one out of the three eggs sectioned. This result is sufficient to show that sometimes, if not always, the second polar spindle is formed while the egg is still in the uterus, previous to fertilization; hence the penetration of the egg by the spermatozoon is not required as a stimulus to the formation of the second polar spindle.

The question arises whether the second polar spindle is normally or ever present after the penetration of the egg by the spermatozoon; in other words, do the processes of maturation and fertilization overlap? We must first take into consideration the possibility that eggs dissected from the uterus of a ripe female for purposes of artificial fertilization may not be quite so far advanced as eggs spawned and fertilized in a natural manner. Fortunately it has been possible to check results obtained through artificial fertilization by comparison with a case in which fertilization occurred in a more natural manner. For the study of fertilization three females, (*C*, *D*, and *E*), were principally used; eggs from the gravid uteri of the first two were artificially fertilized in the usual way; the third female spawned with a ripe male while the two were being carried in a pail of water.

Furthermore we must distinguish between what might be called potential fertilization, the mere contact of the seminal fluid with the gelatinous envelopes of the eggs, and actual fertilization, the penetration of the egg proper by the spermatozoon. While the act of fertilization is not consummated until the fusion of the germ-nuclei, the influence of the spermatozoon is felt in many ways as soon as it enters the egg cytoplasm, so that actual fertilization may be said to begin as soon as the spermatozoon pierces the cell wall of the egg. The time record is almost necessarily reckoned from the moment of mixing of the two sexual elements, or potential fertilization; actual fertilization follows after an interval necessary for the passage of the spermatozoon through the gelatinous envelope, which varies for the individual eggs and especially for eggs of different spawnings fertilized by different males, and which can be determined only by a careful microscopical examination of serial sections of each egg.

Out of twenty-one eggs from three females (*C*, *D* and *E*), preserved at intervals extending from fifteen minutes to two and one-half hours after fertilization, a second polar spindle was found in eighteen cases, and one or more spermatozoa were found in each of eleven eggs. The sections show that the spermatozoon may pierce the cell wall of the egg as early as fifteen minutes after contact with the outer envelopes, though a longer time is usually required.

Making allowance for faults of technique we may say that the second polar spindle is usually and probably always present from the time of fertilization up to two and one-half hours later, reckoned from the moment of mixing the sexual elements; there is no essential difference in this respect between eggs artificially and naturally fertilized.

Only early stages of the second polar spindle are found in eggs up to and including one and one-half hours after fertilization; exclusively anaphase stages are found in eggs taken one and three-quarters to two hours after fertilization; the formation of the second polar body and the egg-nucleus (see figs. 39 and 40) is confined to a period between 4 and 8 hours after fertilization. While a stimulus from the spermatozoon is not required to initiate the formation of the second polar spindle, it is evident that the later stages of this mitosis are passed through only after fertilization; in other words, the processes of maturation and fertilization overlap. Hertwig ('06) makes the general statement that in nature the time of fertilization of the amphibian egg falls between the formation of the first and second polar spindles.

4. *The organization of the egg immediately before fertilization*

At the time of spawning the egg is surrounded by the unchanged zona pellucida or vitelline membrane; within this is a thin cell wall, the transformed zona radiata, which is organically connected with the egg.

There are few changes in the general appearance of the blastodisc since the condition described shortly after the rupture of the germinal vesicle (see fig. 31). There is a more intimate incorporation of the materials of the germinal vesicle into the substance of

the blastodisc; shreds of non-formative material, such as fragments of the nuclear wall and the fibrous material of the germinal vesicle, are each surrounded by a closely adherent film of cytoplasm and are being absorbed. In eggs ready for fertilization the second polar spindle is sometimes, though perhaps not always, fully formed; it lies beneath a sharply-defined pit at the bottom of which may be found the débris of the first polar body.

The peripheral zone of fine yolk particles in the vegetal hemisphere remains as described in the late ovarian egg.

C. FERTILIZATION

1. *The history of the egg-nucleus*

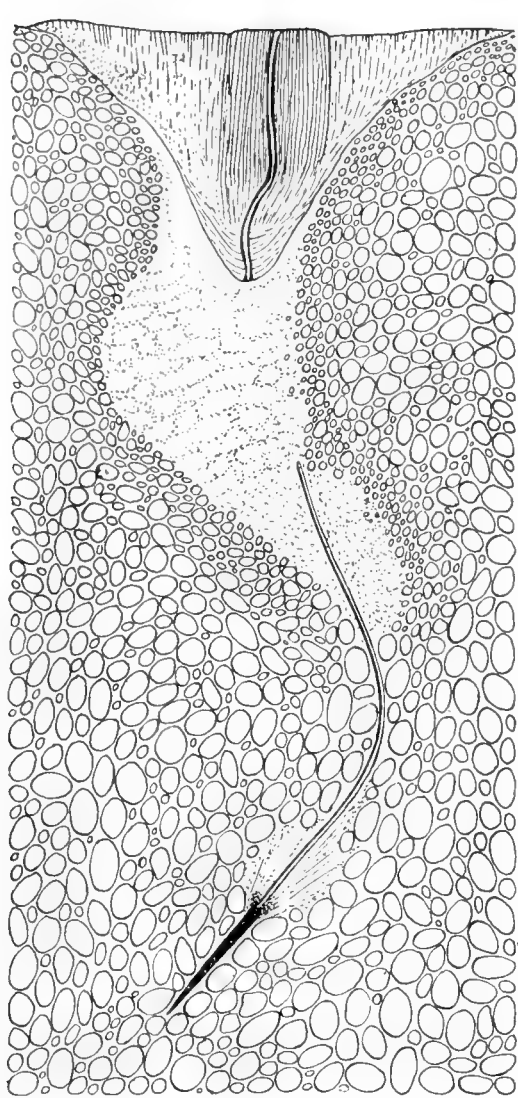
The formation of the egg-nucleus is shown in figures 38 to 40; the process is usually complete about five hours after fertilization. About ten and one-half hours after fertilization (see figs 47 and 48) the egg-nucleus has increased in size and sunk into the blastodisc to a point one-third as far from the surface as the position later occupied by the copulation-nucleus (see fig. 52). A yolk-free region, partly filled with cytoplasm, extends from the egg-nucleus for a short distance toward the surface, indicating the path of migration (fig. 48). At this time the egg-nucleus stains but faintly.

Figs. 41 to 43 Vertical sections of eggs of *Cryptobranchus alleganiensis*, showing penetration of the egg by the spermatozoon. $\times 240$.

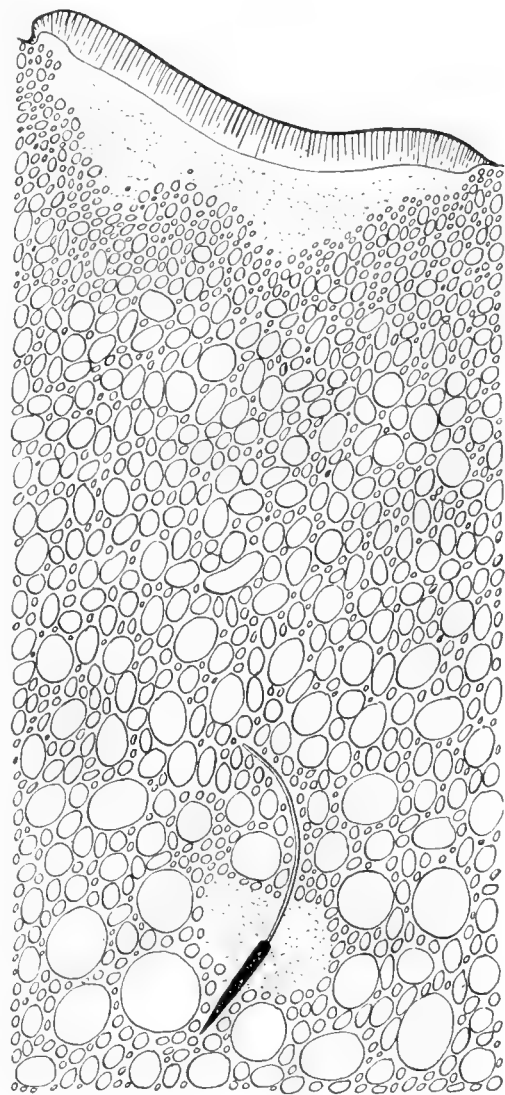
Fig. 41 From an egg killed $2\frac{1}{2}$ hours after fertilization. This figure is a reconstruction from two adjacent sections: the upper half of the figure is drawn from one section, the lower half from the other. The spermatozoon shown in the figure has entered the egg about 50° from the animal pole where the second polar spindle, shown in fig. 38, is in the late anaphase stage. Another spermatozoon in the same condition as the one figured has entered the opposite side of the egg a little below the equator.

Fig. 42 From an egg killed 3 hours after fertilization. The spermatozoon figured has entered the egg a little above the equator. This egg contains in all ten spermatozoa.

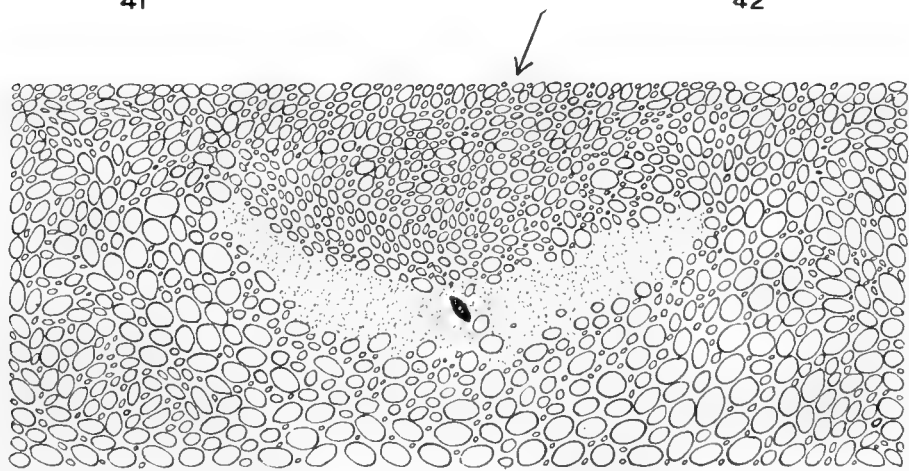
Fig. 43 From an egg killed 5 hours after fertilization. The arrow indicates the direction of the path of the spermatozoon which has entered the egg about 30° from the animal pole. The distance from the surface of the egg to the head of the spermatozoon is about one and one half times as great as in the preceding figures. The head of the spermatozoon is shown entire in this section; the tail persists in a somewhat abbreviated condition, but is not shown in the section figured. This egg contains another spermatozoon in the same condition.



41



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43

2. *History of the sperm-nucleus*

(a). *Penetration of the egg by the spermatozoon.* As previously noted, spermatozoa may be found entering an egg taken as early as fifteen minutes after fertilization. In describing the process, we may best begin with an egg taken about two and one-half hours after fertilization (see fig. 41).

The zona pellucida or vitelline membrane is not affected further than by the formation of a minute perforation which can only rarely be found in sections. The zona pellucida is omitted in the figures.

The cell wall of the egg becomes greatly thickened around the perforation made in it by the spermatozoon. The thickened region is conical in form, with the apex of the cone pointing inward; its outer and central portions are cross-striated. The perforation persists as a conspicuous pore lying in the axis of the cone. The entire structure greatly resembles a micropyle.

Beneath this pseudo-micropyle the path of the spermatozoon is clearly indicated by a yolk-free cytoplasmic region. The form of this region, and the attitude assumed by the spermatozoon itself, indicate that the course pursued by the spermatozoon is a spiral one, with the axis of the spiral lying in a radial direction.

The spermatozoon at this time retains practically its normal form. As in *Axolotl* (Fick, '93) and *Bufo* (King, '01), the tail is not left behind at the surface; in *Cryptobranchus* it continues to serve as an efficient organ of propulsion. The undulating membrane persists, though it is not shown in the figure. The head at this time stains very faintly with the nuclear stain. The acrosome and middle-piece, always difficult to see with the magnification employed for the study of thick serial sections, have not been observed in this situation.

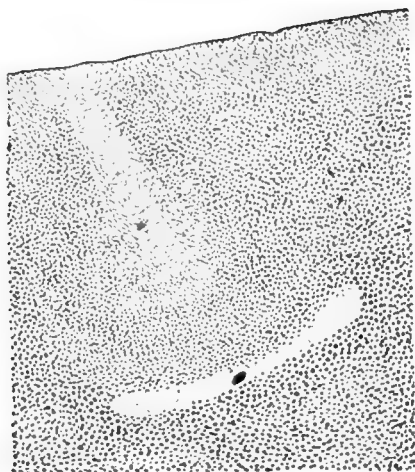
Surrounding the shaft of the spermatozoon for a short distance behind the head there is a spindle-shaped yolk-free region containing cytoplasm. This cytoplasm is particularly dense about the region of the middle-piece; from this locality as a center cytoplasmic strands, resembling linen threads, but finely granular, radiate in all directions, but those extending backward are more

prominent. This phenomenon is much more marked in some other cases than in the one figured.

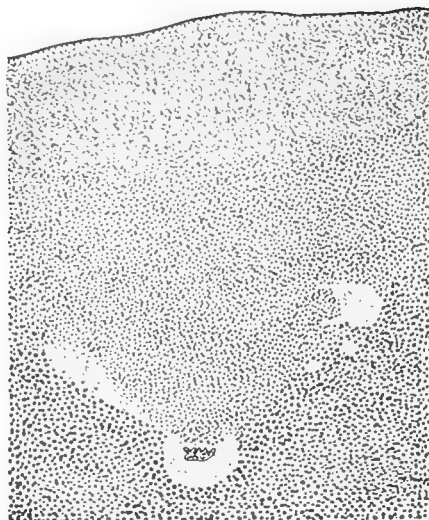
In eggs taken about three hours after fertilization (see fig. 42), the thickening of the cell wall has flattened to the form of a disc; it is strongly striated, recalling the zona radiata from which it takes its ultimate origin. The perforation made by the spermatozoon has disappeared. The cytoplasmic path of the spermatozoon has become filled with yolk, except for a broad shallow region underlying the thickening of the cell wall. The head of the spermatozoon has become shorter and thicker, and takes brilliantly the nuclear stain; the tail has become slightly shorter, perhaps by the degeneration of the posterior portion. The radiations of cytoplasm proceeding from the region of the middle-piece have disappeared, but in the same locality there is a somewhat larger spherical region of uniformly distributed yolk-free cytoplasm.

Five hours after fertilization (see fig. 43), the spermatozoon has penetrated only a little deeper into the egg. The thickening of the cell wall of the egg at the place of entrance of the spermatozoon has disappeared, but its site is marked by convolutions in the cell wall. The protoplasmic path leading from the surface of the egg to the spermatozoon has almost entirely disappeared, but traces of it persist at intervals along the route. The head of the spermatozoon is spindle-shaped and much shorter and thicker than before; the tail persists, but is somewhat abbreviated. The circular area of cytoplasm surrounding the head of the spermatozoon has expanded to form a large crescent, whose horns extend nearly at right angles to the path of the spermatozoon. The yolk granules underlying the crescent are decidedly coarser than those above it. This suggests a correlation of the internal structure with the 'sperm area' seen from the surface: the horns of the crescent produced would meet the margin of the sperm area (compare figs. 9 to 11 with figs 43 to 45).

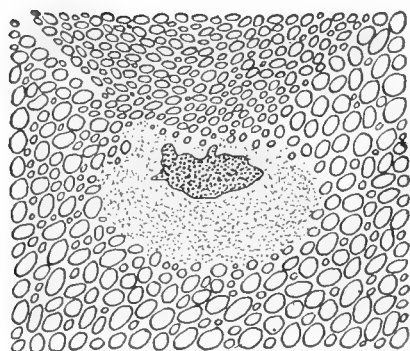
Seven and one-half hours after fertilization (see fig. 44) the protoplasmic path is marked only by a region of sparsely distributed yolk granules extending from the surface for about two-thirds of the distance to the spermatozoon. The yolk granules are particularly dense in the region immediately above the crescent, and



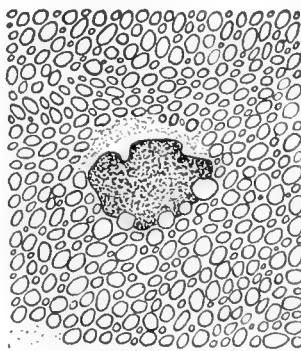
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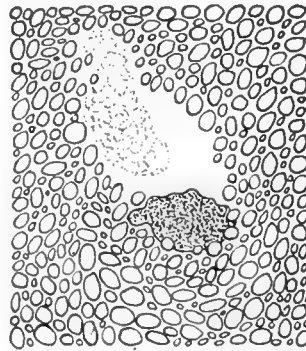
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48

Fig. 44 Vertical section through an egg of *Cryptobranchus allegheniensis* killed $7\frac{1}{2}$ hours after fertilization, showing a late stage in the penetration by the spermatozoon. $\times 80$. This spermatozoon has entered the egg about 30° from the animal pole, and its path inclines toward the axis of polarity of the egg. The head of the spermatozoon is shown entire; the tail persists in an abbreviated and perhaps fragmented condition, but does not appear in the section figured. An aster is present in an adjacent section at a little higher level than the sperm head. Another spermatozoon in the same general condition is found in the same egg.

Fig. 45 Vertical section through an egg killed $10\frac{1}{2}$ hours after fertilization, showing the sperm-nucleus. $\times 80$. The spermatozoon has entered the egg about 25° from the animal pole. Fragments of the tail of the spermatozoon are to be found in the vicinity of the sperm-nucleus, but are not shown in this section.

Fig. 46 A portion of fig. 45 enlarged to show the sperm-nucleus. $\times 240$.

Figs. 47 and 48. Two consecutive meridional sections through an egg of *Cryptobranchus allegheniensis*, killed $10\frac{1}{2}$ hours after fertilization, showing the egg-nucleus. This nucleus is situated about one-third as far from the surface as the copulation-nucleus shown in fig. 52. $\times 240$.

are here finer than elsewhere at the same level. The crescent has become larger, and thicker at the ends than in the middle. The head of the spermatozoon is shortened to the form of a thick spindle and stains deeply; the tail persists in an abbreviated condition. In the case of the spermatozoon shown in figure 44, an aster is found at a slightly higher level than the sperm head and a little nearer to the egg-nucleus. A study of the protoplasmic paths of the aster and the sperm-head shows that they have separated at a point midway in the path of the latter.

Ten and one-half hours after fertilization (see figs. 45 and 46) the spermatozoon has become transformed into the sperm-nucleus, which is amoeboid in form; the tail of the spermatozoon is represented only by fragments. At this time the sperm-nucleus lies about half as far from the surface as the copulation-nucleus shown in fig. 52. Immediately beneath the sperm-nucleus lies a considerable mass of cytoplasm, perhaps formed at the expense of the crescent which is dwindling except at the extreme ends. The remains of the crescent, and the characteristic appearance of the surrounding yolk, enable one readily to distinguish the sperm-nucleus from the egg-nucleus. The sperm-nucleus is smaller than the egg-nucleus, and like the latter does not stain deeply at this time.

The cytoplasmic changes in the egg caused by the invasion of the spermatozoon may be tentatively interpreted as follows: Under the influence of the centrosome, whose seat appears in this case to be in the middle-piece, egg-cytoplasm collects about the neck of the spermatozoon. Here the centrosphere and eventually the entire aster is formed. As the spermatozoon invades the deeper region of coarser yolk particles, the resistance offered to the progress of the accompanying mass of cytoplasm causes it to flatten out like a bullet fired against a wall, assuming a form crescent-shaped in section. Presently the spermatozoon, during its transformation into the sperm-nucleus, comes almost to a full stop, allowing the mass of cytoplasm again to assume a spherical form.

Numerous observers have described in both invertebrates and vertebrates a rotation of the sperm head after it enters the egg,

whereby the middle-piece is brought into position to precede during the further process of migration. Fick ('93) has described this process in *Axolotl*, and Dean ('06) has noted it in *Chimaera*. King ('01) found no indication of a rotation of the sperm head in *Bufo*; possibly this condition is correlated with the fact that in *Bufo* the centrosome appears to be located, not in the middle-piece, but in the head of the spermatozoon. In *Cryptobranchus* rotation of the sperm head apparently takes place at a rather late stage in the process of transformation into the sperm-nucleus. In the stages shown in figures 43 and 44, the greatly shortened sperm head is usually placed with its long axis oblique or at right angles to its former path, so that one end points toward the egg nucleus. But in these stages it has not been possible to trace any connection between the tail of the spermatozoon and its head, and since the aster has already separated from the sperm head, in no case can it be stated which end of the sperm head is the one pointed toward the egg-nucleus.

The spermatozoon ordinarily enters the blastodisc in a more or less centripetal direction, and continues in this direction for a considerable distance; sometimes its path inclines almost from the beginning in an oblique direction toward the point of future union with the egg-nucleus. In either case the axis of the spiral path is ordinarily straight up to the time of the transformation of the head of the spermatozoon into the sperm nucleus; the later course of migration has not been followed. In an egg preserved an hour and three-quarters after fertilization, a spermatozoon, which had entered the blastodisc unusually near the animal pole, described a path which proceeded in a centripetal direction only a very short distance, then curved sharply in a direction parallel to the surface, toward the second polar spindle which was in the late anaphase condition. The form of the spermatozoon remained unaltered, and rotation of the sperm head had not commenced. This case is instructive in showing that the factors tending to bring the germ-nuclei together are active at a very early stage of fertilization: the egg-nucleus was not fully formed, and the spermatozoon had not begun its process of transformation into the sperm-nucleus. Moreover it is evident that in this case at least the

'copulation path' (see Hertwig, '06, p. 529) is not dependent upon the rotation of the sperm head.

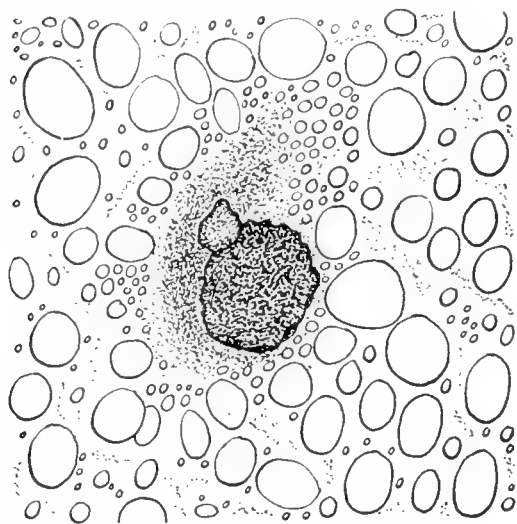
(b). *Polyspermy, and the fate of the supernumerary spermatozoa.* Brief data regarding the occurrence of polyspermy have already been given. It is possible that the method of artificial fertilization increases the number of spermatozoa entering the egg; but in nature the eggs are fertilized in a confined space, and I see no reason to doubt that polyspermy is a common occurrence under natural as well as artificial conditions. It is evident that we have here to deal with physiological, not induced or accidental polyspermy (see Brachet, '10), for the eggs develop in a normal manner.

While the distribution of spermatozoa entering the egg is largely if not entirely a matter of chance, the location in which a spermatozoon finds itself has much to do with its ultimate fate. Spermatozoa entering the lower hemisphere, especially in the region of the vegetal pole, never penetrate far, and since they are found in this hemisphere only during the first few hours after fertilization, must quickly degenerate. In the urodele *Hynobius*, Kunitomo ('10) found that a spermatozoon entering at the vegetal pole sometimes succeeds in reaching the egg-nucleus; but the careful study of many eggs has convinced me that this never occurs in the heavily yolk-laden and strongly telolecithal egg of *Cryptobranchus*.

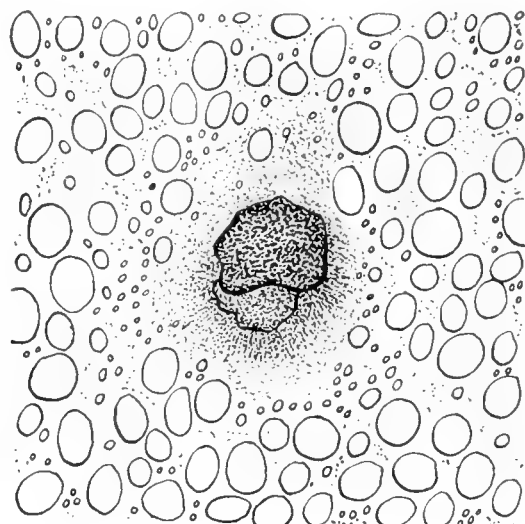
Only in the blastodisc have spermatozoa been found in the stage characterized by the presence of the cytoplasmic crescent (figs. 43 and 44). Obviously, the conditions elsewhere are unfavorable for the formation of any considerable mass of cytoplasm about the spermatozoon. In the stage with a well-formed cytoplasmic crescent, not more than two spermatozoa have been found in a single egg. No accessory spermatozoa have been found in any situation after the formation of a sperm-nucleus. The supernumerary spermatozoa thus have but a transient existence, and the only advantage resulting from polyspermy is doubtless that, in an egg so large, penetration by several spermatozoa is of value in insuring fertilization.

The literature on the occurrence of polyspermy in the amphibian egg has recently been reviewed by Kunitomo ('10). As noted in a previous paper (Smith, '11), I have found polyspermy

occurring in eggs of *Amblystoma tigrinum* fertilized under natural conditions; the material secured for the further investigation of this subject has not yet been worked up. Polyspermy seems to be characteristic of heavily yolk-laden eggs lacking a preformed micropyle.



49



50

Fig. 49 Meridional section through an egg of *Cryptobranchus alleganiensis*, killed 12 hours after fertilization, showing fusion of germ-nuclei. The sperm-nucleus is probably the smaller one. $\times 240$.

Fig. 50 Meridional section showing fusion of germ-nuclei in another egg killed 12 hours after fertilization. The sperm-nucleus is probably the lower and smaller one. $\times 240$. For the position of this copulation-nucleus in the blastodisc see fig. 52 which is drawn from the same section.

3. *Union of the germ-nuclei, and formation of the first cleavage spindle*

In two eggs killed twelve hours after fertilization, the germ-nuclei have been found in the process of uniting (figs. 49 and 50); in these two cases the copulation-nuclei are at approximately the same distance from the surface (see fig. 52), quite deeply situated in the blastodisc and a little to one side of the axis of polarity. The smaller germ-nucleus is probably the sperm-nucleus. The egg-nucleus stains brilliantly with borax-carmin; the sperm-nucleus takes the stain less deeply. The sperm-nucleus especially is surrounded with dense cytoplasm; in one case (fig. 50) this

exhibits a tendency toward radial striation and probably represents the aster.

The study of the paths of migration of the germ-nuclei and the copulation-nucleus is not quite complete, but indicates that the germ-nuclei come together at a higher level than that occupied by the copulation-nuclei shown in the figures.

The first segmentation nucleus in a resting condition has been found in an egg killed eighteen hours after fertilization; the first cleavage spindle has been found in an egg killed seventeen hours after fertilization.

4. *Changes in the blastodisc*

In eggs taken from fifteen minutes to ten and one-half hours after fertilization, cytoplasm is accumulating in irregular patches underlying the animal pole (fig. 51). During this period, practically all traces of the débris of the germinal vesicle disappear. In places, the surface of the blastodisc is sometimes very irregular, almost villous; this may be due to injuries resulting from the actual or attempted entrance of spermatozoa.

In eggs taken from twelve to eighteen hours after fertilization (copulation nucleus to first cleavage spindle) the cytoplasm is gathering in a broken layer close to the surface of the blastodisc. The beginning of this process is shown in figure 52. In *Hynobius*, Kunitomo ('10) has noted a somewhat similar condition. During the latter part of the period considered the layer of cytoplasm becomes much thicker than is shown in the figure, but retains its segmented character.

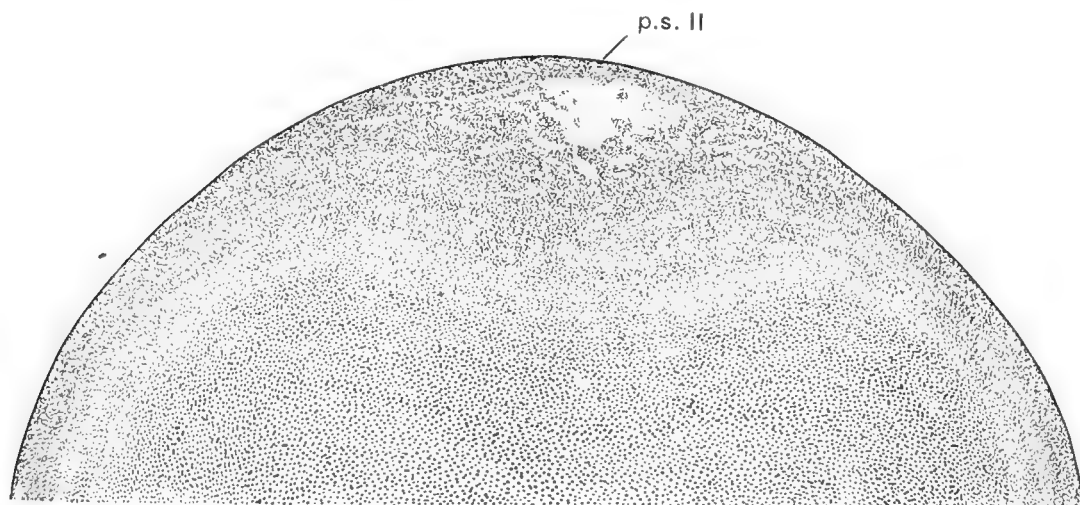
During the first two hours after fertilization there is a marked increase in the thickness and extent of the blastodisc as a whole (see especially fig. 51). Evidently the greater part of this change takes place before the egg has become oriented with the animal pole uppermost, hence it is independent of any possible sorting effect of gravity acting on the materials of the egg.

No marked changes have occurred in the lower hemisphere since the egg left the ovary.

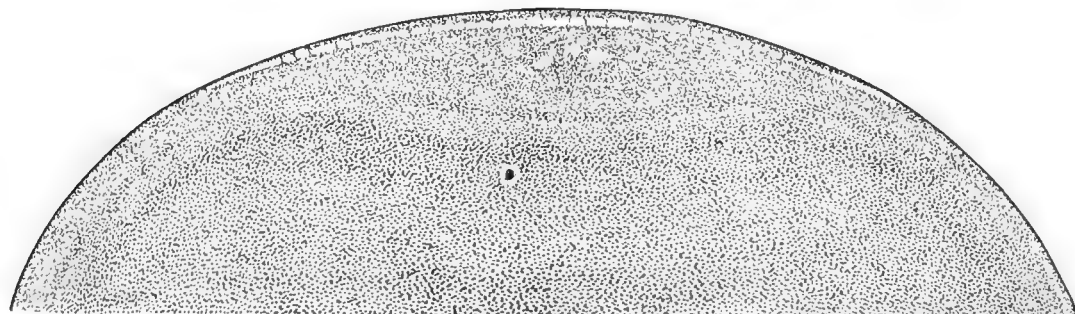
The later changes in the blastodisc lead up to first cleavage and will be considered in that connection.

D. SUMMARY

The follicular layer proper of the ovarian egg of *Cryptobranchus* is formed from some of the deeper non-germinal cells of the ovarian wall which resemble the epithelial cells of the outer and inner limiting membranes. The follicular membrane proper completely surrounds the egg and is suspended in a two-layered flask-shaped sac which projects from the inner surface of the wall of the ovary



51



52

Fig. 51 Meridional section through an egg of *Cryptobranchus allegheniensis*, killed $1\frac{3}{4}$ hours after fertilization, showing the condition of the blastodisc. The irregular faintly stippled areas near the animal pole contain yolk-free cytoplasm. $\times 18$. *p. s. II*, second polar spindle.

Fig. 52 Meridional section through an egg of *Cryptobranchus allegheniensis*, killed 12 hours after fertilization, showing condition of the blastodisc and position of the copulation-nucleus. Yolk-free cytoplasm is segregated in a broken layer near the surface of the blastodisc. The copulation-nucleus is shown a little to the left of the center of the figure. $\times 18$.

into the central cavity; in a broad sense, the entire three-layered structure may be called the follicle.

The zona radiata is formed from the peripheral substance of the egg proper; at the time of the rupture of the germinal vesicle it becomes transformed into a simple cell wall, in organic connection with the egg.

The zona pellucida is formed as a secretory product of the follicular layer proper; it persists unchanged as the 'vitelline membrane' of the embryo.

The earliest observed phenomena which may perhaps indicate polarity occur in the ovarian eggs of young females of a body length of 26 to 30 cm., as a shifting of the region of most abundant vitelline bodies from the future vegetal to the future animal hemisphere. In the ovarian eggs of young females of a body length of 35 cm. there is a concentration of nucleoli on the side of the germinal vesicle toward the future animal pole; this may perhaps afford a second indication of polarity.

Yolk-formation begins in the most advanced ovocytes of young females with a body length of 35 cm.; the yolk is first laid down in concentric zones. With respect to the position of the germinal vesicle, the distribution of cytoplasm, and the size of the yolk particles in the different zones, the egg exhibits radial symmetry until after it is nearly or quite filled with yolk.

About the time when the egg becomes completely filled with yolk, the germinal vesicle migrates from its central position toward a point on the surface which is thus defined as the animal pole. Coincident with the migration of the germinal vesicle, axial differentiation of the cytoplasmic and yolk contents of the egg lead to the formation of a germinal disc in the region of the animal pole.

In general the animal pole of the egg lies within the stalk of the follicle and toward the periphery of the ovary.

In the late ovarian egg a structure called the yolk cup is interpreted as the physiological equivalent of the concentric layers of dense fine yolk found in the eggs of birds and various other vertebrates.

Shortly before maturation the germinal disc is temporarily differentiated into two layers: a thin peripheral layer of yolk-

free cytoplasm, and underlying this a thicker layer of especially fine yolk particles rich in cytoplasm. Both layers are continuous with much thinner layers of the same character surrounding the remainder of the egg.

In the ovocyte ready for maturation, the germinal vesicle lies close to the surface at the animal pole, and is surrounded by the germinal disc. A mass of cytoplasm has accumulated beneath the germinal vesicle during the later stages of its migration. The arrangement of materials is now quite strongly telolecithal.

Shortly before the rupture of its wall, the germinal vesicle appears at the very surface at the animal pole. The rupture of the germinal vesicle takes place just before the egg leaves the ovary; the cytoplasmic and yolk layers of the blastodisc now mingle, and the materials of the germinal vesicle, together with the cytoplasm brought with it from the interior of the egg, are incorporated into the blastodisc.

Absorption of degenerating ovocytes is accomplished by means of the follicle cells, which reverse their usual rôle as nurse cells of the egg, and function as phagocytes.

The first polar spindle is formed about the time the egg leaves the ovary, and disappears about the time the egg enters the uterus. There are marked size differences in the chromosomes.

The second polar spindle is formed shortly after the egg enters the uterus; it lies beneath a deep pit readily visible from the surface.

The penetration of the egg by the spermatozoon is not required as a stimulus to the formation of the second polar spindle.

The late stages of the second maturation division, culminating in the formation of the second polar body and the egg-nucleus, are passed through only after the spermatozoon has entered the egg; in other words, the processes of maturation and fertilization overlap.

A structure resembling a micropyle is formed in the cell wall of the egg around the perforation made by the entrance of the spermatozoon. The influence of the entering spermatozoon upon the egg is shown by characteristic changes in the distribution of the yolk and cytoplasm.

Physiological polyspermy is of normal occurrence. The supernumerary spermatozoa lead but a transient existence.

Union of the germ-nuclei takes place at a point deeply situated near the center of the blastodisc, and is followed by the segregation of masses of cytoplasm forming a broken layer near its surface.

BIBLIOGRAPHY

- BRACHET, A. 1910 La polyspermie experimentale comme moyen d'analyse de la fécondation. *Archiv f. Entw.*, Bd. 30, no. 1.
- BRAUER, AUGUST 1897 Beiträge zur Kenntniss der Entwicklungsgeschichte und der Anatomie der Gymnophionen. *Zool. Jahrb. Anat.*, Bd. 10.
- BUDGETT, J. S. 1899 Notes on the batrachians of the Paraguayan Chaco, with observations upon their breeding habits and development, especially with regard to *Phyllomedusa hypochondrialis* Cope. *Quart. Jour. Micros. Sci., N. S.*, vol. 42; also Budgett memorial volume (Kerr, '07 a).
 1901a On the breeding habits of some west African fishes, with an account of the external features in the development of *Protopterus annectens*, and a description of the larva of *Polypterus lapradei*. *Trans. Zool. Soc. London*, vol. 16, part II, August. Also Budgett memorial volume (Kerr, '07 a.)
 1901b The habits and development of some west African fishes. *Proc. Camb. Philos. Soc.*, vol. 11; also Budgett memorial volume (Kerr, '07 a).
- BÜHLER, A. 1902 Rückbildung der Eifollikel bei Wirbeltieren. I. Fische. *Morphol. Jahrb.*, Bd. 30.
- DEAN, BASHFORD 1896 The early development of *Amia*. *Quart. Jour. Micros. Sci.*, vol. 38.
 1906 Chimaeroid fishes and their development. Carnegie Institution of Washington.
- DEBUSSY, L. P. 1904 Eerste Ontwikkelungsstadien van *Megalobatrachus maximus* Schlegel. *Tijdschrift der nederlandse Dierkundige Vereeniging*, Bd. 8.
 1905 Die ersten Entwicklungsstadien des *Megalobatrachus maximus*. *Zool. Anz.*, Bd. 28.
- EYCLESYMER, ALBERT C. 1906 The habits of *Necturus maculosus*. *Amer. Nat.*, February.
- FICK, R. 1893 Ueber die Reifung und Befruchtung des Axolotleies. *Zeitschr. f. wiss. Zool.*, Bd. 56.
- HARGITT, CHAS. W. 1888 Recent notes on *Scaphiopus holbrookii*. *Amer. Nat.*, vol. 22.
- HARRINGTON, N. R. 1899 Respiratory and breeding habits of *Polypterus*. *Science, N. S.*, vol. 9, pp. 314-315.
 1899 The life habits of *Polypterus*. *Amer. Nat.*, vol. 33, p. 721.

- HAY, O. P. 1888 Observations on *Amphiuma* and its young. *Amer. Nat.*, vol. 22.
 1890 The skeletal anatomy of *Amphiuma* during its earlier stages. *Jour. Morph.*, vol. 4.
- HERTWIG, OSCAR 1906 *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere*. Bd. 1, Th. 1. Jena, Gustav Fischer.
- ISHIKAWA, C. 1904 Beiträge zur Kenntniss des Riesen-Salamanders (*Megalobatrachus maximus* Schlegel). *Proc. Dept. Nat. Hist., Tokyo Imper. Mus.*, vol. 1, no. 2.
- JENKINSON, J. W. 1909 *Experimental embryology*. Oxford. Clarendon Press.
- KERBERT, C. 1904 Zur Fortpflanzung von *Megalobatrachus maximus* Schlegel. *Zool. Anz.*, Bd. 27, no. 10.
- KERR, J. GRAHAM 1900. External features in the development of *Lepidosiren*. *Phil. Trans. Royal Soc., Ser. B*, vol. 192.
 1901 The development of *Lepidosiren paradoxa*. II. With a note upon the corresponding stages in the development of *Protopterus annectens*. *Quart. Jour. Micros. Sci., N. S.*, vol. 45.
 1907 a The work of John Samuel Budgett. Cambridge. Univ. Press.
 1907 b The development of *Polypterus senegalus* Cuv. Budgett memorial volume (Kerr, '07 a).
- KING, HELEN DEAN 1901 The maturation and fertilization of the egg of *Bufo lentiginosus*. *Jour. Morph.*, vol. 18, no. 2.
 1902 The follicle sacs of the amphibian ovary. *Biol. Bull.*, vol. 3.
 1905 The formation of the first polar spindle in the egg of *Bufo lentiginosus*. *Biol. Bull.*, vol. 9, no. 2.
 1908 The ovogenesis of *Bufo lentiginosus*. *Jour. Morph.*, vol. 9, no. 2.
- KINGSLEY, J. S. 1899 *Text-book of vertebrate zoology*. New York. Henry Holt and Company.
- KINGSBURY, B. F. 1895 The spermathecae and methods of fertilization in some American newts and salamanders. *Trans. Amer. Micros. Soc.*, vol. 17.
- KUNITOMO, KANAE 1910 Ueber die Entwicklungsgeschichte des *Hynobius nebulosus*. *Anatom. Hefte, Beitr. u. Ref. z. Anat. u. Entw.*, Bd. 40.
- LILLIE, FRANK R. 1908 *The development of the chick*. New York. Henry Holt and Company.
- MCGREGOR, J. H. 1896 An embryo of *Cryptobranchus*. *Proc. New York Acad. Sci.*, Dec. 14. Abstract in *Zool. Anz.*, Bd., 20, p. 29.
 1899 The spermatogenesis of *Amphiuma*. *Jour. Morph.*, vol. 15, Supplement.
- OSBORN, H. F. 1888 A contribution to the internal structure of the amphibian brain. *Jour. Morph.*, vol. 2, p. 51.
- PEARL, RAYMOND 1909 The nature of the stimulus which causes a shell to be formed on a bird's egg. *Science*, March 12.
- PIKE, NICHOLAS 1886 Notes on the hermit spadefoot (*Scaphiopus holbrookii* Harlan). *Bull. Am. Mus. Nat. Hist.*, vol 1, no. 7.

- REESE, ALBERT M. 1903 The habits of the giant salamander. Pop. Sci. Mo., April.
 1904 The sexual elements of the giant salamander, *Cryptobranchus allegheniensis*. Biol. Bull., vol 6, no. 5.
- REIGHARD, JACOB 1903 The natural history of *Amia calva* Linnaeus. Mark anniversary volume.
- RIDDLE, OSCAR 1911. On the formation, significance and chemistry of the white and yellow yolk of ova. Jour. Morph., vol. 22.
- ROUX, W. 1883 Ueber die Zeit der Bestimmung der Haupttrichtungen des Froschembryo. Leipzig.
 1885 Ueber die Bestimmung der Haupttrichtungen des Froschembryo im Ei und über die erste Theilung des Froscheies. Bresl. ärzt. Zeitschr.
 1887 Die Bestimmung der Medianebene des Froschembryos durch die Kopulationsrichtung des Eikernes und des Spermakernes. Archiv f. mikros. Anat., Bd., 29.
 1903 Ueber die Ursachen der Bestimmung der Haupttrichtungen des Embryo im Froschei. Anat. Anz., 23.
- SARASIN, P. B. and C. F. 1887-1893 Zur Entwicklungsgeschichte und Anatomie der ceylonischen Blindwühlen, *Ichthyophis glutinosus*. Ergebnisse naturwissenschaftlicher Forschungen auf Ceylon, Bd.2.
- SASAKI, C. 1887 Some notes on the giant salamander of Japan. Jour. Coll. Sci., Imper. Univ. Tokyo, Japan, vol. 1, part III.
- SCHULTZE, O. 1900 Ueber das erste Auftreten der bilateralen Symmetrie im Verlauf der Entwicklung. Archiv für mikr. Anat., Bd. 55.
- SMITH, BERTRAM G. 1906 Preliminary report on the embryology of *Cryptobranchus allegheniensis*. Biol. Bull. vol. 11, no. 3.
 1907 The breeding habits of *Amblystoma punctatum*. Amer. Nat., vol. 41, no. 486, June.
 1907 The life history and habits of *Cryptobranchus allegheniensis*. Biol. Bull., vol. 13, no. 1, June.
 1908 The spawning habits of *Chrosomus erythrogaster* Rafinesque. Biol. Bull., vol. 14, no. 6, May.
 1910 The structure of the spermatophores of *Amblystoma punctatum*, Biol. Bull., vol. 18, no. 4, March.
 1911 The nests and larvae of *Necturus*. Biol. Bull. vol. 20, no. 4, March.
 1911 Notes on the natural history of *Amblystoma jeffersonianum*, *A. punctatum*, and *A. tigrinum*. Bull. Wis. Nat. Hist. Soc., vol. 9, April.
- TOWNSEND, CHAS. H. 1882 Habits of *Menopoma*. Amer. Nat., vol. 16, no. 2.
- WIEDERSHEIM, R. 1900 Brutpflege bei niederen Wirbeltieren. Biol. Centralb., Bd. 20.
- WHITMAN, C. O. 1898 Animal behavior. Woods Hole Biological Lectures.

PLATE 1

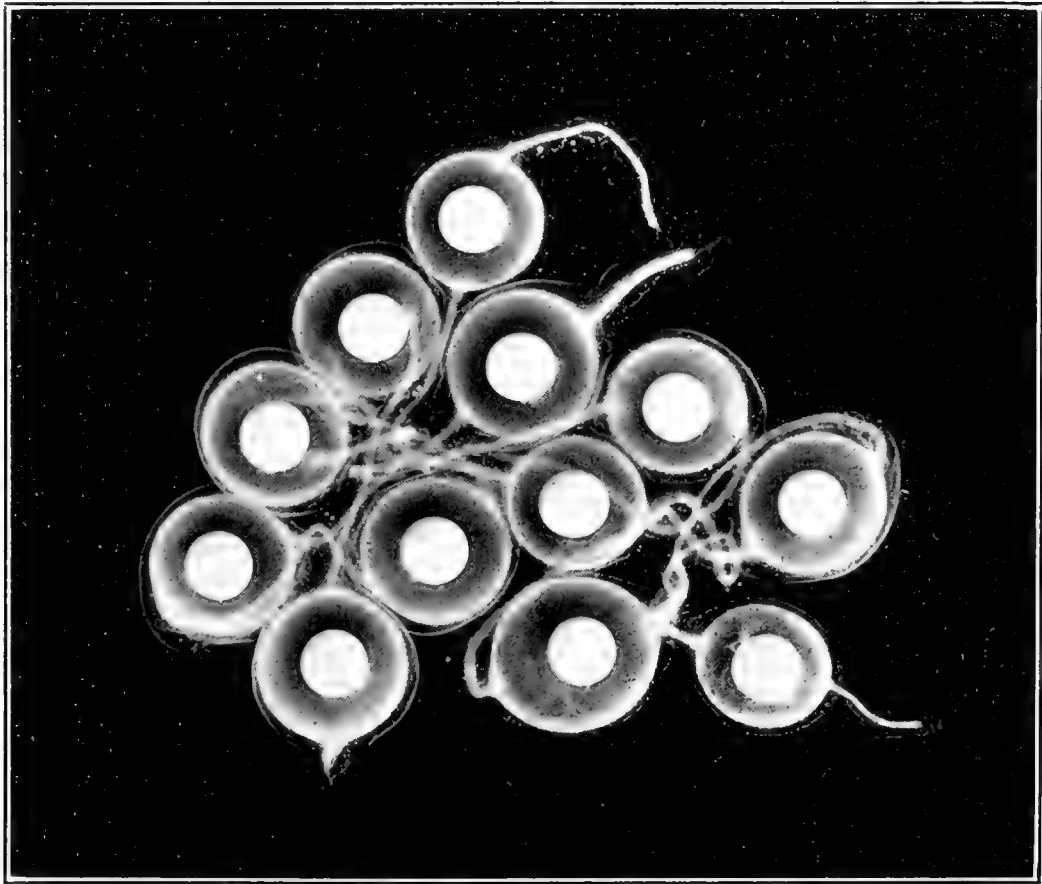
EXPLANATION OF FIGURES

53 *Cryptobranchus allegheniensis*. Living eggs dissected from the ovary, showing the germinal vesicle at the surface. The small spots within the germinal vesicle are probably nucleoli. Ovarian membranes containing blood-vessels wholly or partially cover the eggs. $\times 4$.

54 *Cryptobranchus allegheniensis*. Unfertilized eggs with their gelatinous envelopes, after two days' immersion in water. Natural size.



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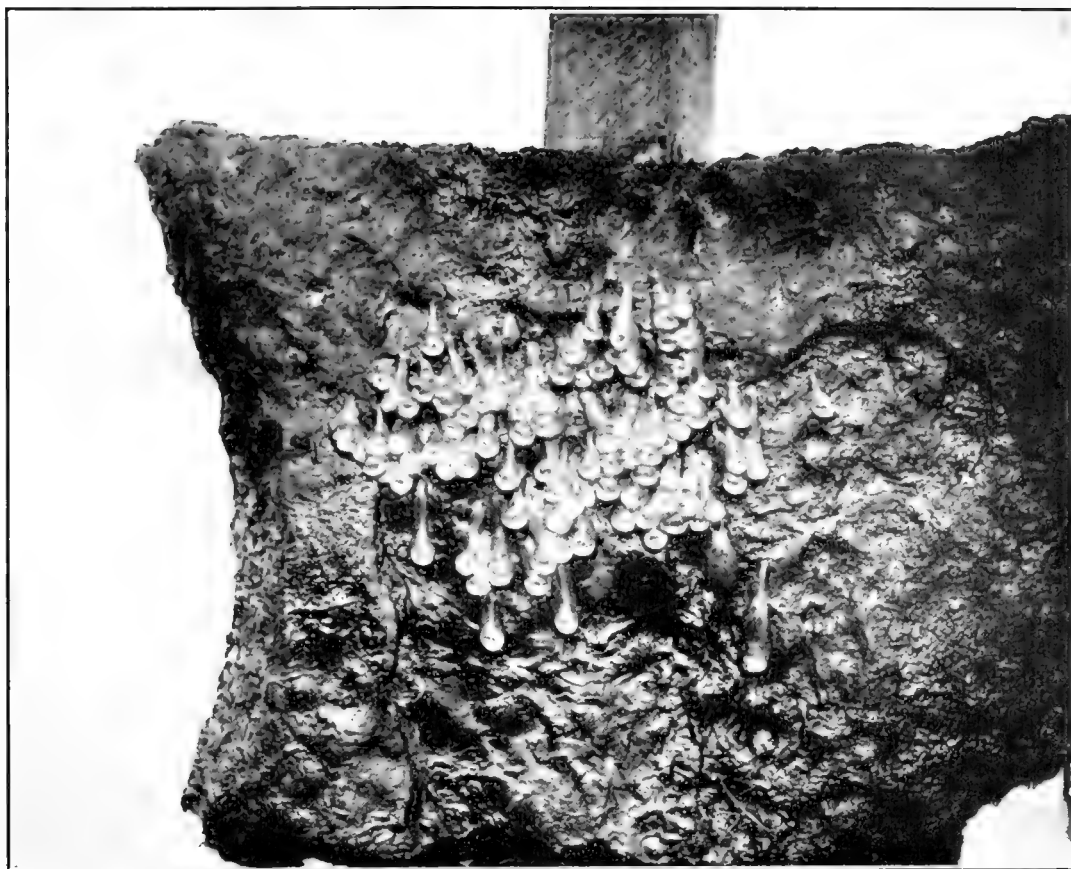
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PLATE 2

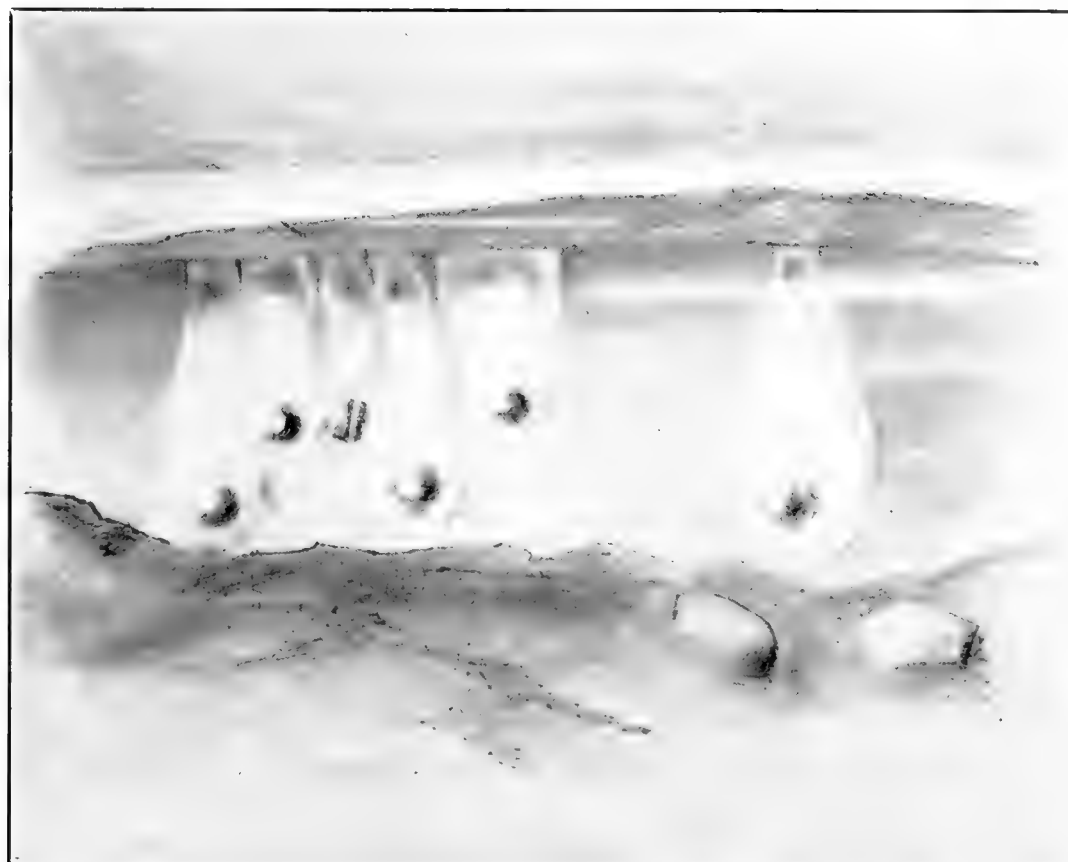
EXPLANATION OF FIGURES

55 *Necturus maculosus*. 'Nest' of eggs of *Necturus*. The stone to which the eggs are attached has been removed from the water and set on edge on the wharf; it is about 16 inches in diameter. The embryos are in an advanced stage of development.

56 *Necturus maculosus*. Eggs and envelopes shown in their natural position in the water, attached to a piece of board; natural size. From a drawing by Professor Bashford Dean.



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BODY SIZE AND CELL SIZE¹

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TWELVE FIGURES

In his very thoughtful and suggestive address at the Zoological Congress of the World's Columbian Exposition on "The Inadequacy of the Cell Theory of Development" Professor Whitman ('93) pointed out the fallacy of the view, prevalent since the time of Schleiden and Schwann, that "organization means cellular structure and that ontogeny means cell formation." On the other hand he maintained that "organization precedes cell formation," and that "the secret of organization, growth, development lies not in cell formation, but in those ultimate elements of living matter for which *idiosome* seems to me an appropriate name."

The truth and importance of this position were well supported in his argument and have been justified by later work on the organization of the germ cells, as well as by the older work on the Protozoa; but no one would have been more ready than Professor Whitman to recognize the fact that this protest against a dominant doctrine did not express the whole truth. It may be granted that a certain amount of organization precedes cell formation without granting that all organization does. Indeed we know that the latter is not true; very much of the organization which we see in higher organisms occurs only after cell formation, and, in all probabilities, as a result of it. Indeed the organization which is possible without cell formation is probably only such as is found among Protozoa and germ cells.

What the full meaning of cell formation in the development of higher organisms is we do not know, but it seems practically

¹ Prepared for The Whitman Memorial Volume, but received too late to be included.

certain that it is associated not merely with growth in size but also with growth in complexity. The degree of differentiation of an organism is not determined entirely by the number of cells in its body, but cell number is certainly one of the factors in differentiation. It is now known that the protoplasm of certain egg cells is composed of different substances which may be localized in different parts of the egg, and in this respect it resembles the protoplasm of a protozoan cell. Lillie ('02) has shown that certain simple differentiations, such as the appearance of cilia, may occur in eggs which do not undergo cleavage, but these differentiations do not go beyond this primitive, protozoan stage. When these differentiated portions of the egg protoplasm are separated from one another by cell membranes, the differentiations increase in degree, if not in number. The whole progress of embryonic differentiation is thus associated with differential cell division.

Of course there are many cell divisions which are non-differential, in which the daughter cells are alike, and such cell divisions are not essential to progressive differentiation, though they are essential to growth. We are thus able to separate growth and differentiation, for while the latter consists in an increase in the kind of structures, the former consists in an increase in their size and number merely. In embryonic development, however, these two processes usually go hand in hand; and in many instances size differences between cells are the earliest differentiation which can be recognized.

Almost all species of animals and plants have a more or less characteristic body size which, in spite of individual variations, may be said to constitute the norm of the species. Specific differences in body size may be slight, or they may be enormous, as in the case of the mouse and the elephant. In similar manner different individuals of the same species may differ in a marked degree in body size, the individuals which represent the extremes of size being known as dwarfs and giants. The question whether the difference between a dwarf and a giant, or between a small sized and a large sized race or species, is due to a difference in size of the ultimate units of structure, the cells, or to a difference

in their number, or to both, is one of fundamental interest and importance.

Some of the earliest observations in this field were made by botanists and served to show that differences in body size are due to the number of cells present rather than to the size of the individual cells. Erich Amelung ('93) determined "that the larger or smaller development of a plant body has no influence on the size of its adult constituent cells." Sachs ('93) also called attention to the lack of correlation between cell size and body size. Strasburger ('93) reached the same conclusion concerning the embryonic cells from the growing points of extremely large and small individuals. He says: 2

Not the cell size, only the cell number, is influenced by the different size of an individual It was surprising to me to find that while individuals of the same species always showed the same size of embryonic nuclei and cells, varieties of these same species might differ greatly from each other.

In an interesting paper on one of the mutants of *Oenothera lamarckiana*, *O. gigas*, Gates ('09) comes to practically the same conclusion. He says (p. 543):

In *O. gigas* we have an organism built of bricks which are larger and whose relative dimensions are also altered in some cases. These two factors will apparently account for all the differences between *O. gigas* and *O. lamarckiana*, and the second factor may be one merely of readjustment consequent upon the first. It is probable that the number of cells is approximately the same in both cases.

This is evidently one of those cases, of which Strasburger speaks, in which different species or varieties of the same species may differ greatly from each other in cell size.

In the case of animals the earlier work on cell size was confined largely to studies on nerve cells and fibers. Gaule ('89) concluded from a study of the spinal cord of the frog that differences in the size of individuals of the same species influenced only the size of the ganglion cells but in no way influenced their number, which might be considered a constant factor. Donaldson ('95) after summarizing a number of observations on the number and size of nerve cells in man says (p. 192):

The determination of the number of neuroblasts occurs so early in the history of an individual, and under such uniform conditions, that it is very difficult to regard the environment as possessed of much power to cause variation in this respect, and for this reason among members of the same race a high degree of constancy in this character is to be anticipated.

He regards differences in brain weight as due to differences in the size of the nerve cells, the number remaining constant. Hardesty ('02) found that in various mammals the motor nerve cells from the spinal cord were largest in the elephant and smallest in the mouse, while in animals which were intermediate in size the nerve cells were intermediate. Levi ('05) has made an extensive study of cell size in different species, particularly mammals. He finds that the size variations of epithelial and gland cells are insignificant and are not correlated with body size. Measurements of ganglion cells gave entirely different results; here the size of the cell varies with the size of the animal. Nerve fibers and lens fibers show the same correlation with body size as do ganglion cells. In muscle he finds that the diameter of the fiber is usually larger in large animals than in small ones, though this is subject to many variations. Levi points out the significant fact that epithelial and gland cells divide throughout life, whereas the other types of cells named cease to divide at an early age.

Morgan ('95) concluded that in echinid larvae derived from isolated blastomeres the number of cells is approximately proportional to the size of the blastomeres or egg fragments from which they came. However in the case of partial larvae of *Amphioxus* Morgan ('96) held that one-half larvae contained about two-thirds as many cells as whole larvae, while one-fourth larvae had more than one quarter, but not quite one-half the total number in a whole larva. Driesch ('98, '00) determined in a very satisfactory manner that the number of cells in partial larvae of echinids is one-half the normal number in one-half larvae, one-fourth in one-quarter larvae, etc., while in larvae from two fused eggs the number of cells is double the normal number. And since these partial or double larvae all have the typical structure of a normal larva he was led to the formulation of the 'rule of the fixed size of specific organ-cells.'

Rabl ('99) found that the size of cells of the crystalline lens was practically constant, but the cell number a variable one, depending upon the size of the organ. Boveri ('04) also found that in dwarfs and giants of the human species the size of epithelial cells from the tongue and of bone corpuscles from a phalanx agreed perfectly with those from an individual of normal size.

Chambers ('08), on the other hand, has questioned the view that a certain cell size is characteristic of a species or race; he finds that the size of an individual frog, and the size of its constituent cells, depends upon the size of the egg from which it came. In agreement with the earlier work of Morgan ('04), he has found that the size of the frog's egg may vary considerably; whether laid by the same individual, or by different ones, the diameters of eggs of extreme size may vary as much as 1 : 3. The smaller eggs give rise to smaller tadpoles and frogs, which are composed of smaller cells, than those derived from larger eggs. He believes with Popoff ('08) that the initial cause of the variations in the sizes of eggs is to be found in unequal division of the nucleus or plasma of the oocytes.

Popoff ('08) holds that sperm cells as well as egg cells vary in size. He supposes that when a large egg is fertilized by a large spermatozoon a large individual with large cells results; whereas from small eggs and small spermatozoa small individuals with small cells arise. He admits that the operations of this law may be obscured by two other conditions, viz., (1) various factors which limit or inhibit growth, and (2) rich nourishment which influences only the number of cells, and not their individual size. He affirms, therefore, that body size is not inherited as commonly supposed—it has no specific representative in the germ plasm; only the size of cells, not the size of individuals, is inherited.

Berezowski ('10) has made a study of cell size in relation to body size in white mice, varying in age from ten days to five months, and in body volume from 4 cc. to 25 cc. He finds that with the growth of an animal there is an increase in the cell size and nuclear size, particularly in the length of cells and of nuclei of the intestinal epithelium adjoining the pylorus. In a second paper,

Berezowski ('11), shows that the cell size is slightly greater in castrated mice than in normal ones.

In a series of brilliant studies, Jennings ('08, '09, '10, '11, etc.) has shown that at least eight different races, or 'pure lines,' of *Paramecium* may be distinguished by their size. The mean length of the largest race is to that of the smallest about as 5 : 1. While the variations in length within each race is considerable, the norm for each race is quite characteristic. He finds that the differences in size between individuals of the same race are due to growth and environment and are not inherited, whereas the differences between different races are inherited.

In addition to the foregoing references there are doubtless many other observations on the relations of cell size to body size scattered through the literature. These references, however, are believed to include the most important works on this subject, as well as the principal conclusions which have been reached.

The following observations on the relative size and number of cells from the bodies of different species of *Crepidula*, and from different individuals of the same species were made many years ago, and a brief note on this subject was published at that time (Conklin '96), and a somewhat more complete account in a subsequent paper ('98).

1. CELL SIZE AND BODY SIZE IN DIFFERENT SPECIES OF CREPIDULA

I have made no extensive study of cell size in relation to body size in different classes of animals, most of my work having been confined to different species of gasteropods, and principally to the genus *Crepidula*. Without any special study on this subject, however, it is quite evident from casual observation that different classes differ widely in cell size, and that these differences are not usually correlated with differences in body size. The great size of cells in amphibians, nematodes and some insects is well known, whereas in echinoderms, annelids and mammals the cells are relatively much smaller. Even different species, and varieties of the same species, may show considerable differences in cell size, as Strasburger, Gates and Boveri have shown.

a. Body size. I have studied in some detail the body size and cell size of four species of the genus *Crepidula*, as well as of several species of other prosobranch gastropods. The mature females of *Crepidula* are always larger than the males and are firmly attached to some object from which they are unable to move; the males on the other hand are smaller and have greater power of locomotion. In the following measurements of body size and cell size the two sexes were always carefully separated; twenty mature individuals of the same sex were chosen and, after having been removed from their shells, they were placed upon blotting paper to remove any excess of water and were then

TABLE 1

Body size in the genus Crepidula

SPECIES	ACTUAL VOLUME OF BODY		RELATIVE VOLUME IN SAME SPECIES OF		RELATIVE VOLUME IN DIFFERENT SPECIES	
	Male	Female	Male	Female	Male	Female
	cc.	cc.				
<i>C. convexa</i>	0.01	0.05	1	5.0	1.0	1.0
<i>C. adunca</i>	0.025	0.208	1	8.3	2.5	4.16
<i>C. plana</i>	0.046	0.667	1	14.5	4.6	13.34
<i>C. fornicata</i>	1.25	1.60	1	1.34	125.0	32.00

dropped into a known volume of water in a graduated tube; in this way the average body volume of the twenty specimens was determined.

b. Tissue cells. Various tissue cells from corresponding organs and parts of organs of *C. convexa*, *C. plana*, and *C. fornicata* have been measured with the Zeiss 1/1 micrometer eyepiece and 3 mm. homogeneous immersion objective. The average dimensions of such cells are given in table 2, and these measurements show plainly that there is no constant correlation between body size and cell size in these different species; the size of many tissue cells is practically the same in all species (e.g. most of the epithelial cells) while in those cases where the cell size differs in different species, the larger cells are not always found in the species of larger body size. Certain cells vary so much in size, especially

in large organs where it is impossible to be certain that precisely corresponding cells have been measured, that no great reliance can be placed upon such measurements and comparisons; this is the case with the epithelium of the mantle, stomach and kidney. On the other hand in organs which consist of relatively few cells, or where the individual cells are easily identified, much confidence can be placed in these measurements; this is the case with the cells of the gill filaments, osphradium, liver ducts, muscles, and sex cells.

TABLE 2

Size of adult tissue cells in different species of Crepidula

VOLUME OF BODY	C. CONVEXA		C. PLANA		C. FORNICATA	
	0.05 cc.		0.65 cc.		1.5 cc.	
	Cell	Nucleus	Cell	Nucleus	Cell	Nucleus
	μ	μ	μ	μ	μ	μ
<i>Tissue cells</i>						
Ectodermal epithelium						
Mantle near anus...	6 x 12	6 x 9	6 x 14	6 x 9	—	—
Gill filament, external ciliated cells...	3 x 18	3 x 6	4 x 15	4 x 8	4 x 15	4 x 7
Cells internal to chitinous rod.....	4 x 12	4 x 6	4 x 15	4 x 9	4 x 12	4 x 6
Osphradium.....	3 x 66	3 x 12	3 x 75	3 x 10	4 x 66	4 x 7
Retina, maximum cells.....	6 x 21	6 x 6	6 x 24	6 x 6	7 x 24	6 x 7
Endodermal epithelium						
Liver duct.....	6 x 21		5 x 24		7 x 21	
Liver cells, without secretion.....	6 x 27		7 x 30		—	
Liver cells, with secretion.....	15 x 50		15 x 59		12 x 33	
Rectum.....	6 x 36		9 x 28		6 x 27	
Mesodermal cells						
Blood corpuscles, maximum.....	6		6		6	
Muscle fibers, maximum diameter.....	10		6		6	

Although the body size differs greatly in different species of *Crepidula*, the volume of an adult male of *C. fornicata* being one hundred and twenty-five times that of the male of *C. convexa*, and the volume of an adult female of *C. fornicata* being thirty-two times that of the female of *C. convexa*, the size of the tissue cells is practically the same in all of these species. Of course it follows that the number of cells is much greater in the larger sized species than in the smaller sized ones.

c. Sex cells. In one type of cell, the sex cells, there is a constant and considerable difference in size between the different species. In all stages of the development of the ova, from the last generation of oogonia to the fertilized egg, these cells differ markedly in size in the different species. In *C. convexa*, *C. fornicata*, and *C. plana* the diameters of the oogonia at the time when they are preparing for their last division are $27\ \mu$, $16\ \mu$ and $12\ \mu$ respectively. Similarly the first generation of oocytes, before the formation of yolk begins, measure $57\ \mu$, $42\ \mu$ and $36\ \mu$ respectively in these three species. These differences in the diameters of the oocytes are associated with corresponding differences in the sizes of their nuclei, yolk nuclei and yolk spherules, as is shown in table 3.

There are also differences in the manner of yolk formation in these three species; in *C. plana* and *C. fornicata* the yolk granules appear to be formed pretty uniformly throughout the protoplasm of the egg; in *C. convexa* the yolk is formed at the base of the cell, where it is attached to the ovarian wall, while the portion of the oocyte next the lumen of the follicle is a cap of protoplasm which for a long time remains free from yolk.

Finally the dimensions of the fertilized but unsegmented eggs of four species of *Crepidula* and one species of *Fulgur*, together with the average number of eggs laid by each mature female, and the total volume of these eggs as compared with the body volume, is given in table 4. There is here no constant relation between the size of the individual egg and the volume of the adult, though in general the species of *Crepidula* of smaller body size produce the larger eggs (v. Conklin, '97). On the other hand the size of the egg is correlated with its mode of development, the

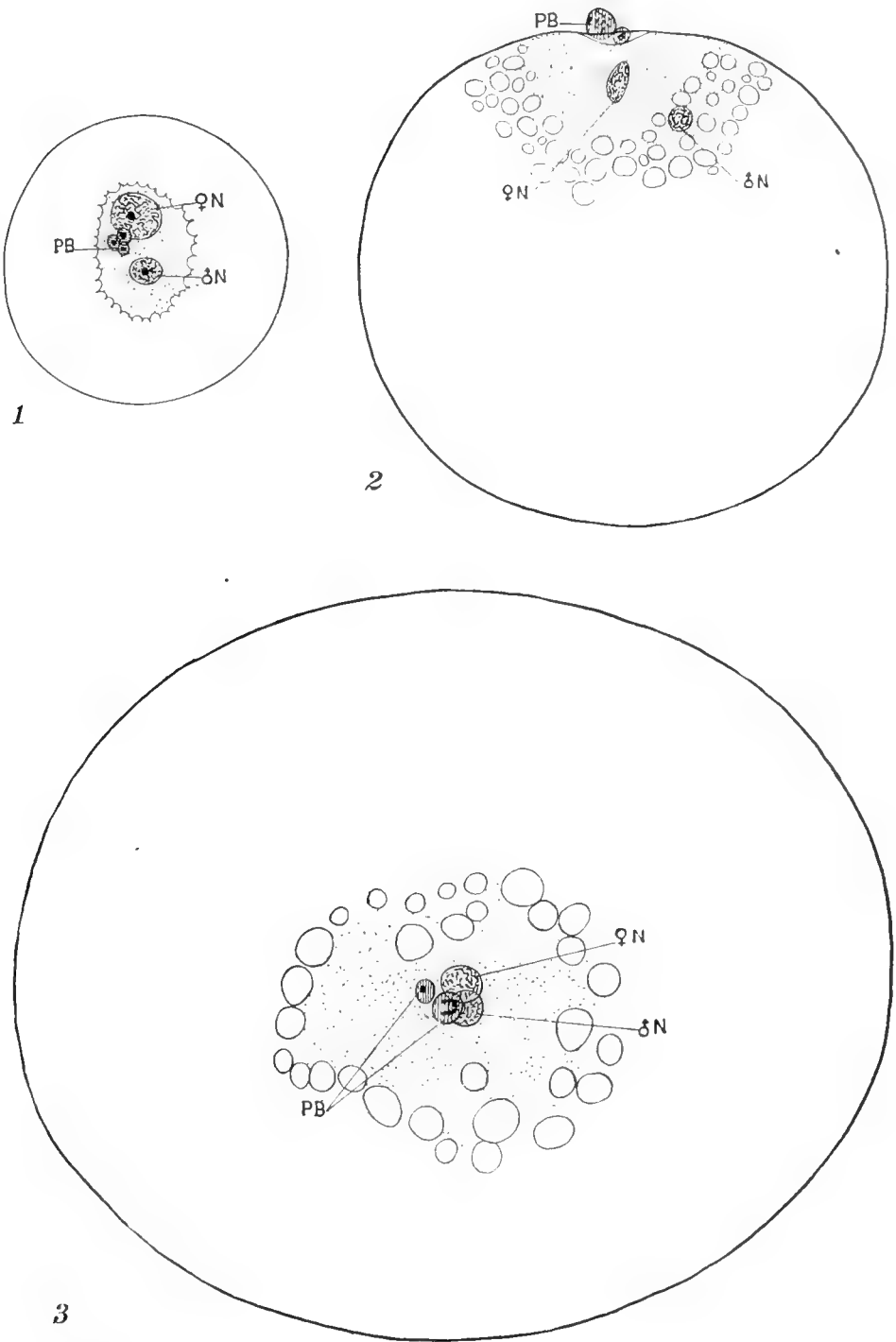
TABLE 3

Size of sex cells in different species of Crepidula

	C. CONVEXA		C. PLANA		C. FORNICATA	
	Cell	Nucleus	Cell	Nucleus	Cell	Nucleus
	μ	μ	μ	μ	μ	μ
Oogonia, at last division.....	27	15	12	6	15	12
Oocytes I, before formation of yolk.....	57	28	36	20	42	24
Oocytes I, maximum yolk nucleus....		27		6		12
Oocytes I, maximum yolk spheres.....		36		15		12
Ootids, after fertilization.....	280		142		182	
Spermatocytes I.....	9	7	8	6		
Spermatocytes II.....	8	6	7	5		
Spermatid, chromatin condensed.....	4	3	3	2		
Mature spermatozoa						
Eupyrene { Head..	24		15		12	
{ Middle						
{ piece	30		30		30	
{ Tail...	110					
Oligopyrene { Length	66		54		54	
{ Width.	1.5		2		2	

smaller eggs producing free-swimming larvae while the larger eggs give rise to larvae which undergo metamorphosis within the egg capsules and escape only when the adult form has been reached.

The larger eggs have a much larger quantity of yolk than the smaller ones, but they also have a larger quantity of cytoplasm and larger nuclei; even in the oogonial stages, before any yolk is formed, these cells are much larger in *C. convexa* than in *C. plana*; indeed these oogonia differ as much in size as do the mature eggs. It would be a great mistake to suppose that the larger eggs differ from the smaller ones merely in the quantity of yolk which they contain, as is usually assumed. Such a marked and constant difference in the size of egg cells, where other types of cells are so uniform, is significant. There is some evidence that in the species



Figs. 1 to 3 Unsegmented eggs of *Crepidula plana*, *C. convexa*, and *C. adunca*, drawn to the same scale, to show relative sizes of eggs, germ nuclei, and cytoplasmic and yolk areas.

with the larger eggs a considerable number of oogonia, or of follicle cells, fuse to make a single egg. In the very early stages of the oogonia, in *C. convexa*, there is no marked difference between the germ cells and the follicle cells; later they differentiate and the follicle cells become more numerous than the oogonia. Both follicle cells and oogonia are sometimes imbedded in young oocytes, or even in the oogonia, and in such cases the size of the oocytes is greater and their number fewer than in other species in which such a fusion has not been observed.

TABLE 4

Number, size and volume of eggs as compared with volume of adult female

SPECIES	NUMBER EGGS LAID	DIAMETER EGG	VOLUME EGG	TOTAL VOLUME EGGS	VOLUME ADULT ♀	RATIO VOLUME EGGS TO VOLUME ♀
		μ	cu. mm.	cu. mm.	cu. mm.	
<i>C. plana</i>	ca. 9000	142	0.001489	13.4	667	1 : 50
<i>C. fornicata</i>	ca. 13200	182	0.003156	41.65	1600	1 : 38
<i>C. convexa</i>	ca. 220	280	0.011494	2.53	50	1 : 20
<i>C. adunca</i>	ca. 180	410	0.036087	6.50	208	1 : 32
<i>Fulgur carica</i>	ca. 750	1600	2.13	1597.5		

The adults of the several species of *Crepidula* are so variable in size, color and form that it is frequently difficult to distinguish the species; however the egg size of each species is highly characteristic and constant, and by this means I have been able to distinguish doubtful species, and in one case to show that a supposed species (*C. glauca* Say) is only a locally modified form of *C. convexa*, (Conklin, '98). I know of no other animals in which the size and form of sexually mature individuals are so variable and the specific egg size so constant as in the genus *Crepidula*.

A similar, though less marked, size difference is seen in the male sex cells of the different species of *Crepidula*. In table 3 the dimensions of the spermatocytes of the first and second order, the spermatids, and the mature spermatozoa, both eupyrene and oligopyrene, are given for *C. convexa*, *C. plana* and *C. fornicata*. In the case of the eupyrene sperm the tail is very long, about 110 μ in *C. plana*, and it is very tenuous toward the end so that I have not been able to measure it with certainty; however it is

easy to get the average length of the head and middle piece in all three species. These measurements show that the male cells at all stages are larger in *C. convexa* than in the other species named, thus forming a parallel case with the egg cells of this species.

Popoff ('08) maintains that there are small variations in the sizes of the sex cells of different species, caused by inequalities of division and by unequal growth during the growth period. He supposes that when a large egg is fertilized by a large spermatozoon a large individual, composed of large cells, results; whereas if the sex cells are smaller than usual the individual developing from them will also be smaller. Applying this hypothesis to the case of *Crepidula*, we should expect to find that *C. convexa*, which has larger eggs and spermatozoa than the other species considered in table 2, would show a larger body size and cell size than the other species; on the contrary the size of tissue cells is not greater, and the body size is much less in *C. convexa* than in *C. plana* and *C. fornicata*. In this case it is evident that the egg size does not determine the body size nor the cell size of the adult, but that differences in body size are due to varying rates of growth and cell division in the different species. It is true that I am here dealing with different species, whereas Popoff's hypothesis applied to different individuals of the same species, but it would be a remarkable fact if so general a proposition as Popoff's should be completely reversed in two closely allied species. We have not generally regarded specific differences as so fundamental.

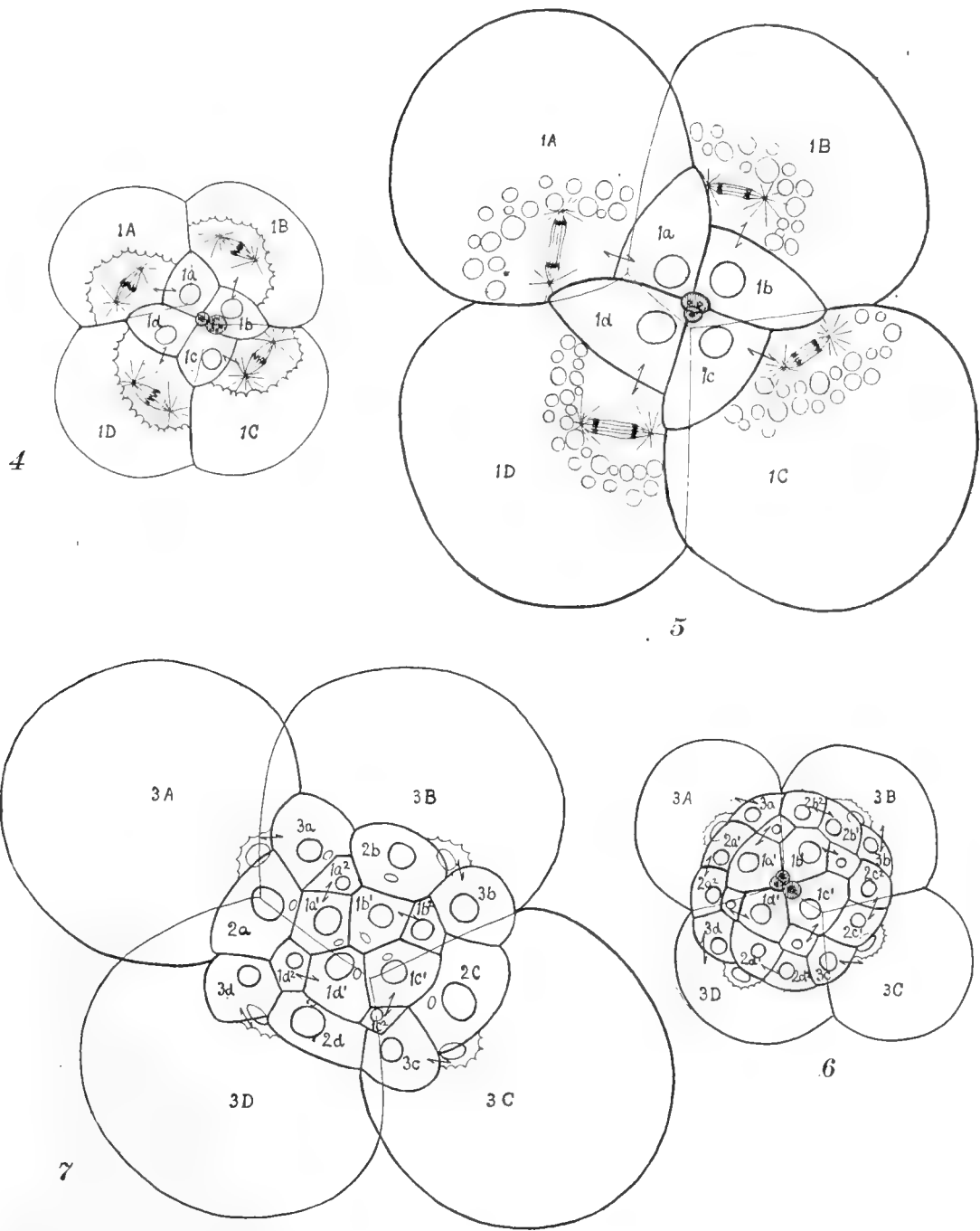
Popoff admits that body size may be the result of conditions favorable or unfavorable to growth. A study of the conditions which lead to the production of dwarfs or giants in *Crepidula plana* shows that here these factors are environmental, and not germinal; I have discussed elsewhere (Conklin '98) this case and will summarize it later in this paper. Undoubtedly environmental conditions have much to do with body size. But in the case of different species, each with characteristic body size, the factors which determine size cannot be merely environmental.

In all animals there is a limit, and in most cases a clearly defined one, to body size and consequently to cell growth and cell division. This limit may be imposed by unfavorable environment, or by

certain intrinsic conditions, which are for the most part unknown. In some instances there is a direct relation between egg size and body size, as in the male and female eggs of *Dinophilus*, phylloxerans, rotifers and spiders (Montgomery '08). On the other hand there is marked dimegaly of the sexes in each species of *Crepidula*, as shown in table 1, without any corresponding dimegaly of the sex cells, but it is possible that protandric hermaphroditism may sometimes occur in these species (Conklin, '98, Orton '09). It is well known that egg fragments produce smaller embryos than entire eggs, and Zur Strassen ('98) has shown that from two fused eggs of *Ascaris megalocephala* a giant individual may result. According to Morgan ('04) and Chambers ('08) frogs' eggs which are smaller or larger than usual give rise to individuals which are smaller or larger than the mean. All of this shows that within a species there may be a relation between body size and the size of the 'Ausgangszellen.' But at the most this is only one factor of several which determine body size, and in many cases, as in the genus *Crepidula*, the other factors are the more important ones.

In the case of different species or varieties, even though closely related, it is evident that egg size in general cannot be directly correlated with body size. Here the rate and duration of cell growth and cell division are the most important factors in determining body size.

d. Blastomeres. In the early cleavage of the eggs of these gasteropods the blastomeres are, cell for cell, the same, except for size, in all the species, whatever the size of the egg may be. The direction of cleavage and its relation to the chief axis of the egg, the rhythm of cleavage and the relative sizes of daughter cells, the constitution of the blastomeres, whether protoplasmic or deutoplasmic, and the ultimate destination of the individual blastomeres is the same in all the species of *Crepidula*, (figs. 4 to 12). In all of them the ectomeres are separated from the entomeres as three quartets of micromeres, which contain most of the cytoplasm of the egg but no yolk (figs. 4 to 7); in all of them the mesomere (4*d*) arises from the left-posterior macromere, and contains both yolk and cytoplasm; in all species, the entomeres are the four



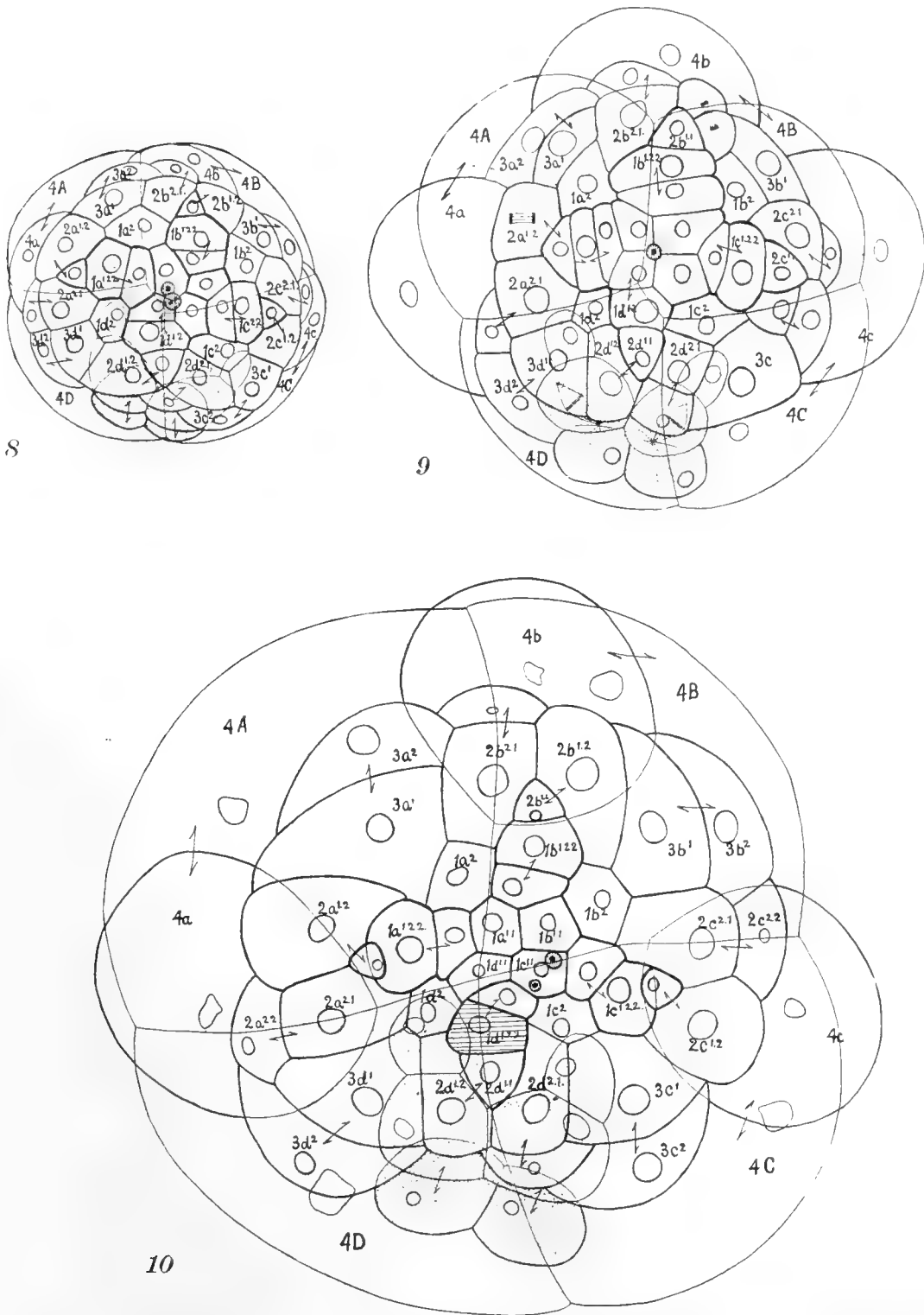
Figs. 4, 5 Eight-cell stage of eggs of *C. plana* and *C. convexa*, drawn to the same scale, showing relative sizes of blastomeres, nuclei, spindles, etc.

Figs. 6, 7 Twenty-four-cell stage of eggs of *C. plana* and *C. convexa*, drawn to same scale and showing relative sizes of blastomeres, nuclei, and protoplasmic and deutoplasmic regions of the egg.

large macromeres which contain little cytoplasm and almost all the yolk. The early subdivisions of the ectomeres take place in exactly the same way in the largest as in the smallest eggs, though the individual cells are larger in the former than in the latter. When the third and last quartet of ectomeres is separated from the macromeres, the first quartet has divided, and, in the smaller eggs of *C. plana*, the second quartet also, so that the completely segregated ectoderm consists of a plate of sixteen, or twenty, protoplasmic cells resting upon the great yolk cells, or macromeres (figs. 6, 7). Since this ectodermal plate contains most of the cytoplasm of the egg, its dimensions in the different species give a fair idea of the relative amounts of cytoplasm in these eggs. This plate is larger in the large eggs than in the small ones, as table 5 shows, and of course the individual cells of which it is composed are larger in the former than in the latter. It will be seen by consulting table 5 that the diameter of the ectodermal plate is considerably greater than the diameter of the cytoplasmic area of the unsegmented egg; this is due in large part to the more complete segregation of the cytoplasm in the later stage than in the earlier one, though in part it is due to the growth of cytoplasm at the expense of yolk, as I have shown elsewhere ('12.)

It is a matter of capital importance that all differential cleavages of the egg are precisely similar in number and character in all these species of *Crepidula*, whatever the size of the egg may be. Not only are all the cleavages which give rise to ectomeres, mesomeres and entomeres the same in all species of the genus, but all subdivisions of these cells, which are differential in character are the same in all these species, so far as I have been able to determine. It is only in the case of non-differential cleavages that differences in the number of cells appear in the different species. But while the differential cell divisions do not vary in number under normal conditions, this number is not to be regarded as irrevocably fixed, for it may be experimentally altered, as I shall show in another paper.

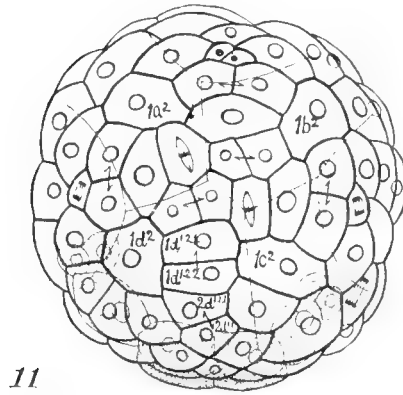
Although the number of cleavage cells is the same in all species during the early cleavage stages, it comes to differ greatly in the



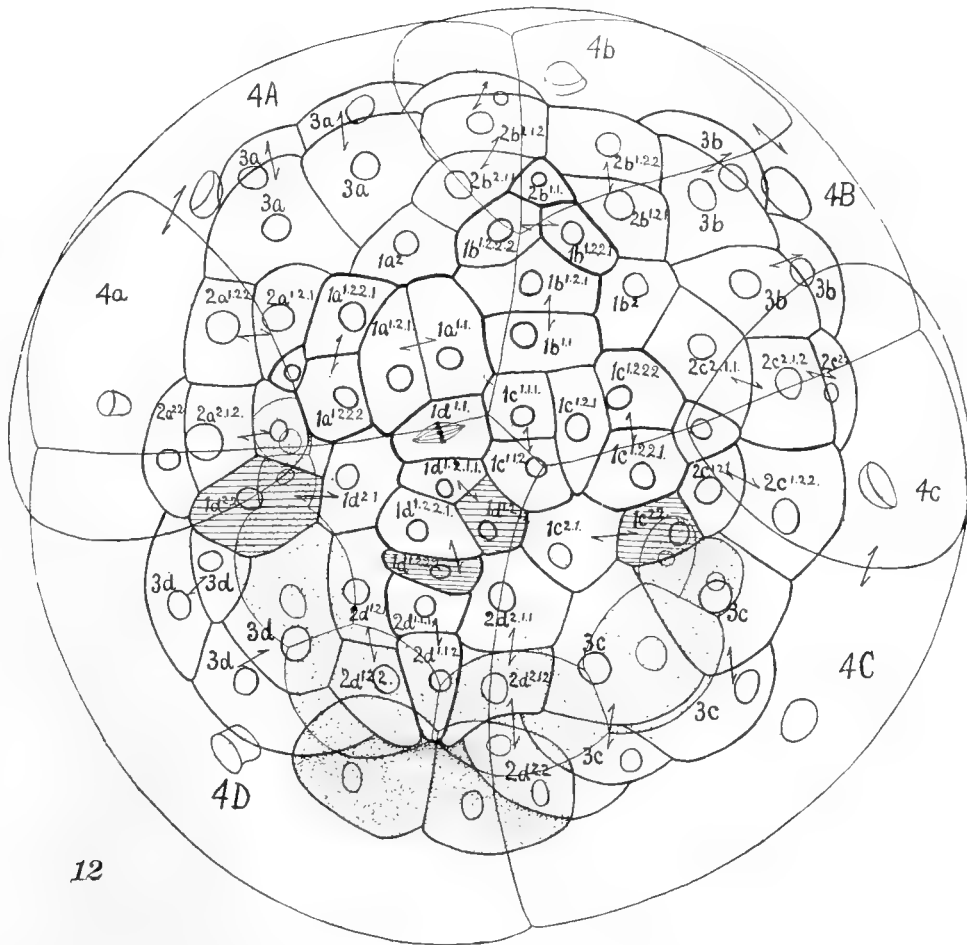
Figs. 8 to 10 Corresponding stages in the eggs of *C. fornicata*, *C. convexa*, and *C. adunca*, drawn to the same scale. The blastomeres correspond cell for cell except that one additional ectoderm cell (the basal cell in the posterior arm of the cross, shaded by transverse lines) is present in *C. adunca* (fig. 10) which is not present in the other species. There are present: 39 ectoderm cells (40 in *C. adunca*), 6 mesoderm cells, 7 endoderm cells.

later stages, the number of ectoderm cells being greater in the larger eggs than in the smaller ones. Up to the 52-cell stage the number of cells is the same in the eggs of all the species examined; at this stage one additional ectoderm cell appears in the posterior arm of the 'cross' in *C. adunca* which does not appear until later in the other species (the additional cell is the one shaded by transverse lines in fig. 10). At the 82-cell stage four such additional cells are present in *C. adunca*, two in the posterior arm of the cross and two in the posterior 'turret' cells, all the other cells being the same in all the species (figs. 11, 12). These additional cells of the posterior arm of the cross and of the posterior turret cells are all similar histologically as well as in 'prospective significance' with the cells from which they were derived. They all become the large ciliated cells of the 'posterior cell plate' (Conklin, '97, p. 109), and ultimately give rise to the large cells which form the head vesicle of the larva. In the later stages of cleavage many additional ectoderm cells appear in the larger eggs which are not present in the smaller ones. Most of these additional cells appear in the primordia of organs after these have been established, and consequently represent only an increase in cells of a given kind. These primordia form chiefly on the oral side of the egg, whereas the greater part of the aboral hemisphere is covered by the large cells of the posterior cell plate. As a consequence the number of cells visible from the aboral pole at the period of the closure of the blastopore does not differ greatly in number in the different species; whereas the cells visible from the oral pole differ greatly in number in the different species, there being more than three times as many in *C. adunca* as in *C. plana* at the time when the blastopore closes, and in later stages this disproportion becomes much greater.

Table 5 gives the diameter of the egg and of the ectodermal plate in different species of *Crepidula*, at the time when the ectomeres are first separated from the macromeres; also the approximate number of ectoderm cells, visible from the oral and the aboral poles at the time of the closure of the blastopore, in the different species.



11



12

Figs. 11, 12 Corresponding stages in the eggs of *C. fornicata* and *C. adunca*, drawn to the same scale. In the former there are present 62 ectoderm, 10 mesoderm and 7 endoderm cells; in the latter 66 ectoderm, 10 mesoderm, and 7 endoderm cells. The 4 additional ectoderm cells in *C. adunca* are 2 additional posterior turret cells and 2 posterior basal cells, shaded by transverse lines.

TABLE 5

Cell size and cell number in the development of Crepidula and Fulgur

SPECIES	1-CELL STAGE		24-CELL STAGE		CLOSURE OF BLASTOPORE	
	Diameter of egg	Diameter of proto- plasmic area	Diameter of egg	Diameter of ectoderm plate	Number of cells visible	
					Oral pole	Aboral pole
	μ	μ	μ	μ		
C. plana.....	ca. 142	ca. 65	ca. 134	ca. 80	164	124
C. fornicata.....	ca. 182	ca. 86	ca. 182	ca. 100	262	140
C. convexa.....	ca. 280	ca. 90	ca. 280	ca. 144	430	146
C. adunca.....	ca. 410	ca. 120	ca. 410	ca. 200	530	164
Fulgur carica.....	ca. 1600	ca. 160	ca. 1600	ca. 320	ca. 5000	?

This table shows that although there are many more ectoderm cells in the larger eggs of *Crepidula* than in the smaller ones, this increased number is chiefly confined to the oral pole, where the primordia of the various organs are located, and it also indicates, as was emphasized above, that the increase in the number of cells in *C. adunca* as compared with *C. plana*, is due to a greater number of non-differential divisions in the former species after the primordia have been established.

It seems probable that the more frequent divisions of the ectomeres in *C. adunca* as compared with those of *C. plana* is associated with their larger initial size, but at present it is not possible to determine why the smaller cells of the latter species continue to grow and divide for a longer period than the larger cells of the former.

e. Larvae. Finally the body size and cell size of fully formed larvae of *C. plana*, *C. convexa*, and *C. adunca* are given in table 6. In the case of *C. plana* the larvae measured were of maximum size and were ready to escape from the egg capsules; in the other species the larvae were of a corresponding stage of differentiation, with velum and larval organs of maximum size, though these larvae undergo metamorphosis within the egg capsules and do not escape until they are adult in form. The body volume of these larvae was roughly determined by measuring the length and breadth of the body as seen from the dorsal side, the thickness of the body being approximately the same as its width.

TABLE 6

Body size and cell size of fully formed larvae of Crepidula

	C. PLANA	C. CONVEXA	C. ADUNCA
<i>Dimensions of body</i>			
	μ	μ	μ
Dorsal view { Length.....	240	400	550
Breadth.....	230	335	480
Relative volumes.....	1	3	10
<i>Dimensions of cells</i>			
Oesophagus.....	6 x 12	9 x 12	6 x 15
Stomach (pyloris).....	6 x 12	7 x 15	9 x 15
Foot epithelium.....	4 x 27	4 x 24	4 x 30
Gill epithelium.....		6 x 12	5 x 15
Retina (post. wall)*.....		6 x 15	6 x 18
Sex cells (?)		5	6

While the relative volumes of the unsegmented eggs of these three species are as 1 : 7 : 24, the relative volumes of the larvae are as 1 : 3 : 10; in short, the growth of the embryo and larva of *C. plana* has been more rapid than that of *C. convexa* and of *C. adunca*, so that the great disproportion which existed between the eggs of these species at the beginning of development, has begun to disappear.

Likewise the cell dimensions of the larvae of these three species show that the great disproportion in size of the early cleavage cells of *C. plana*, as compared with *C. convexa* or *C. adunca*, has begun to disappear. The cells of the larvae of *C. plana* are but little smaller than those of *C. convexa* and *C. adunca*, but they are much fewer in number. Even among the larvae differences in body size are due chiefly to differences in cell number, rather than to differences in cell size, just as is true of adults.

Evidently growth in volume during embryonic stages has been more rapid in *C. plana* than in the other species named. This may be due to a greater absorption of water on the part of the embryo of *C. plana*; and in accordance with this suggestion it may be said that the cytoplasm of this embryo is less dense and stains more faintly than that of the embryos of the other species. Davenport ('97) showed, long ago, that the increase in bulk of the frog embryo and larva, up to the time when it begins

to take food, is due to absorption of water; and in the embryos and larvae of *Crepidula* it seems probable that varying rates of growth may be due to this same factor. This increased growth of the embryo of *C. plana* as compared with those of the other species, continues through the larval and post-larval stages, so that in the end the adults of the first named species become much larger than those of the latter. On the other hand the number of cell divisions during embryonic stages is much greater in *C. convexa* and *C. adunca* than in *C. plana*, so that the embryos and larvae of the former species are composed of a much greater number of cells than in the case of the last named species. But in post-larval stages cell divisions become much more numerous in *C. plana* than in *C. convexa* or *C. adunca*, so that in the adult condition the first named species contains a very much greater number of cells than either of the others.

Considering only the intrinsic factors of growth, one may say that the size of an embryo is the result of the initial size of the egg, but the size of an adult individual is due for the most part to the duration and rate of cell growth and cell division. The size of the germ cells is of little significance in determining the body size of adult individuals in different species of *Crepidula*, though it may possibly be of some importance in determining the body size of different individuals of the same species and race, when all other conditions are equal. In different races and species the important factor in determining body size is the duration and rate of cell growth and cell division. Many extrinsic factors are known to limit or promote such growth and division, but in the case of species which differ greatly in size and in which body size is a very characteristic feature it would seem necessary to suppose that size is inherited,—that some of its causes must be intrinsic ones. What these intrinsic factors are which limit cell growth and division in some cases and which promote such processes in others, is at present entirely unknown. However it seems probable that upon such factors depends in the main the difference between a mouse and an elephant, while the initial size of the sex cells is of only minor importance.

2. CELL SIZE AND BODY SIZE IN TYPICAL AND DWARFED INDIVIDUALS OF *CREPIDULA PLANA*

I have already discussed in detail an interesting case of environmental dimorphism in *C. plana* (Conklin, '96, '97, '98), but since this work has apparently escaped the attention of other workers on this subject I shall here quote from one of these papers ('98) at some length. The ordinary or typical form of

Crepidula plana is found most abundantly in dead shells of *Neverita* inhabited by the large hermit crab, *Pagurus polycarpus*. In this position individuals grow to a large size, mature females frequently reaching a length of two inches and a breadth of one and one-quarter inches. A dwarf race of *C. plana* occurs in the dead shells of *Nassa* or *Littorina*, inhabited by the little hermit crab, *Pagurus longicarpus*; the largest individuals of this race never exceed three-quarters inch in length and three-eighths inch in breadth, i.e., they are about one-third the linear dimensions of the larger form.

There is good evidence that these dwarfs are not a permanent variety or race. In the first place there are no anatomical differences between the two varieties save size only; secondly the eggs, embryos and larvae of the two cannot be distinguished; . . . finally, a few specimens were found which showed by the shape and character of their shells that at one time they had been typical dwarfs; afterwards, having been detached, they obtained a new foothold on a larger surface, and their shells increased in size, the new portions of the shell being shaped so as to fit the surface upon which they had found a new home. In every such shell one can recognize both the dwarf and the normal forms. The dwarfs are what they are by reason of external conditions, and not because of inheritance; they are, in short, a physiological and not a morphological variety.

The average body volume of a mature female of *C. plana* is $\frac{2}{3}$ cc., while the average volume of a mature female of the dwarf variety is $\frac{1}{6}$ cc., i.e., the average body volume of the typical form is about thirteen times that of the dwarf. This disproportion in size would be much greater if comparison were made between the largest individuals obtainable in the two classes, since the dwarfs are much more uniform in size than the type forms.

The dwarfs are perfectly formed in all respects, and all organs of the body seem to be reduced in about the same proportions. It is interesting to note that certain organs, or parts of organs, which are formed in considerable numbers in the course of development, are reduced in number but not in size in the smaller individuals;² this is true of the number

² In agreement with these observations are the experiments of Miss Peebles ('97) on the regeneration of small pieces of hydra; in such cases one, two, three or four tentacles are formed, depending upon the size of the regenerating piece.

of gill filaments, and the number of lobules of the liver and ovary. The number of gill filaments in three individuals, which differed greatly in size, was as follows:

Mature female.....	Volume of body, 0.75 cc., Gill filaments, 204
Immature female.....	Volume of body, 0.05 cc., Gill filaments, 53
Dwarf female (mature)....	Volume of body, 0.05 cc., Gill filaments, 58

Table 7 gives the dimensions of certain tissue cells from sexually mature individuals of *C. plana* of widely different body size. All cells measured are from corresponding parts of similar organs. In each instance the dimensions given represent an average of about one hundred cells from at least four different individuals of approximately the same size. I am indebted to Mrs. Anna N. Bigelow, one of my former students, for the care with which she performed the laborious task of making these many measurements.

With the exception of the ganglion cells and the muscle fibers, the differences in cell size in these different individuals is slight

TABLE 7
Size of tissue cells of mature individuals of Crepidula plana of different body size

	VOLUME OF BODY		
	Giant ♀ 0.75—.8 cc.	Dwarf ♀ 0.05 cc.	Typical ♂ 0.05 cc.
	μ	μ	μ
Ectodermal epithelium			
Mantle, near anus.....	6 x 13	6 x 14	6 x 13
Gill chamber, epithelial lining.....	6 x 11	5 x 11	6 x 12
Foot, epithelium.....	6 x 14	6 x 15	6 x 15
Gill filament, external ciliated cells....	4 x 15	4 x 15	3 x 15
Gill filament, cells internal to chitinous rod.....	3 x 15	4 x 12	4 x 12
Osphradium.....	3 x 75	4 x 75	4 x 78
Ganglion cells, largest in pedal ganglion	17 x 23	9 x 20	10 x 20
Endodermal epithelium			
Liver duct.....	6 x 21	6 x 20	6 x 18
Liver cells, with secretion.....	15 x 59	16 x 58	17 x 66
Stomach opposite liver duct.....	13 x 54	12 x 54	11 x 43
Rectum.....	10 x 28	12 x 27	10 x 25
Mesoderm cells			
Kidney cells.....	13 x 38	16 x 38	13 x 34
Muscle fibers from foot, diameter.....	6	5	5

and insignificant, and these results, which were briefly reported in both of my former papers on this subject ('96, '98), show that differences in the body size of different individuals are due to the number of cells present rather than to the size of individual cells. To quote again from one of the papers referred to ('98, p. 438):

It is an almost impossible task to count the number of cells present even in a very small organ. I have, however, been able to count the number of cells present in cross sections of the rectum, and while the size of the cells here, as everywhere, is the same in the large as in the small individuals the number of cells is greater in the former than in the latter.

Of all the cells of the body, the ova are most easily enumerated; they are laid in capsules which can be easily counted, and each of which contains a nearly constant number of eggs. Oft repeated observation shows that without exception the fertilized, but unsegmented, eggs of the dwarfs are of exactly the same size as those of the giants, but are very much fewer in number; e.g. table 8 shows the averages obtained from a larger number of observations.

It is notable that the number of capsules formed is nearly the same in the two varieties, though there is a great difference in the number of eggs inclosed in each capsule.

In *Crepidula*, therefore, the cell size is fairly constant, and variations in the size of the body are due to variations in the number of cells present.

. Whatever the cause of the dwarfed form may be, it will be noted that in *Crepidula* it operates by stopping growth and cell division.

3. CELL SIZE AND BODY SIZE IN MALES AND FEMALES OF *C. PLANA*

Marked as is the environmental dimorphism in *C. plana*, the sexual dimorphism is even greater (table 1), the average female being almost fifteen times as large as the average male. In all species of *Crepidula* the males are smaller than the females, though the difference in size is greatest in *C. plana*.

TABLE 8

*Size and number of eggs laid by typical and dwarfed individuals of *Crepidula plana**

	DIAMETER OF EGG	NUMBER OF CAPSULES	EGGS IN CAPSULES	TOTAL NUMBER OF EGGS
	μ			
<i>C. plana</i> (type).....	136*	51	176	9,000
<i>C. plana</i> (dwarf).....	136*	48	64	3,070

*More recent measurements, made with another scale and other lenses, show the eggs to be about 142 μ in diameter before the first cleavage, as given elsewhere in this paper.

In the case of the males, as in that of the dwarfs, the smaller size of the body is due to the smaller number of cells present rather than to the smaller size of the cells. Careful measurements of the cells of the intestine, stomach, liver, kidney, muscles of foot, epithelium of gill chamber, epithelium of gill filaments, etc. show that the cell size remains the same in the male as in the female (table 7). Whatever the ultimate cause of the smaller size of the males may be, it operates in this case as in that of the dwarfs, by causing a cessation of growth, and cell division.

It seems probable from the observations of Orton ('10) as well as of myself (Conklin '98) that the small males of the genus *Crepidula* may sometimes grow into the larger females, and that we have here a case of protandric hermaphroditism. If so the smallness of body size and cell number in the males of this genus may be considered to be youthful characteristics.

I have found no evidence that the difference in the size of adult males and females is associated with differences in the size of the eggs as is the case in rotifers, phylloxerans, and *Dinophilus*, and if protandric hermaphroditism occurs in this genus, such dimorphism of the egg would not be expected.

4. CONCLUSIONS

In the genus *Crepidula* differences in body size may be very great; the volume of the average male of *C. fornicata* is one hundred and twenty-five times that of the average male of *C. convexa*; and the volume of the average female of *C. fornicata* is thirty-two times that of the average female of *C. convexa*. Within the single species, *C. plana*, the volume of the average female is about fifteen times that of the average male and about thirteen times that of the dwarf female of the same species.

In spite of these great differences in body size, the size of tissue cells is approximately the same in all species examined, and in all individuals of both sexes and of very different sizes. In the main differences in body size are due to differences in the number of cells present, and not to variations in the size of individual cells. Ganglion cells and muscle cells form the principal exception to this rule.

These results agree with most of the work which has been done on cell size in relation to body size, and particularly with the re-

sults of Levi ('05). On the other hand Berezowski ('10) finds that the size of intestinal epithelial cells is smaller in young mice than in older ones, and that with the general growth of an animal there occurs a growth in the height of these cells. However this observation does not contradict the conclusion reached in this paper; indeed it is true of *Crepidula*, as of the mice studied by Berezowski, that younger animals have smaller cells than older ones, as will be seen by comparing the size of larval cells given in table 6 with that of adult tissue cells given in tables 2 and 7. It is well known that the size of cells depends to a certain extent upon the rate of cell division and the length of the resting period, and the rate of division is slower and the resting periods longer in mature animals than in young ones. In all my measurements I have, so far as possible, compared animals of the same stages, so that the developmental changes in the size of cells does not materially influence my results.

But while tissue cells maintain a very uniform size in *Crepidulae* of all species and sizes, provided they are of corresponding ages, the sex cells differ enormously in size and number in the different species, the species of small body size having in general larger and fewer eggs than species of larger size. On the other hand within the same species the sex cells are of approximately the same size in all individuals, but they differ in number in animals of different body size, just as the tissue cells do.

The larger eggs of *C. convexa* and *C. adunca* are larger in every respect, having more cytoplasm as well as more yolk than the eggs of *C. plana* and *C. fornicata*. Even in the oogonia and early oocytes, before yolk begins to form, the eggs of the former species are larger than those of the latter. The spermatozoa and spermatocytes of *C. convexa* are also larger than those of *C. plana* or *C. fornicata*. Presumably the sex cells of *C. convexa* are larger from the time of their first appearance, and it is possible that this is due to their being derived from larger blastomeres, as well as to the fact that the primitive sex cells divide less often in species with large eggs than in those with small ones. It seems probable also that the oogonia or oocytes engulf a larger number of oogonia and follicle cells in *C. convexa* than in *C. plana*.

The larger eggs give rise to larger blastomeres and to larger embryos and larvae than do the smaller eggs. The size of tissue cells is nearly the same in the larvae of the different species, and this size is less than that of adult tissue cells; but the number of cells in the larvae of different species differs greatly being approximately proportional to the body volume of the various larvae. Finally cell growth and division continue for a longer period in species of *Crepidula* which have small eggs than in those which have large ones, with the result that the former give rise to larger adults than the latter. In the different species of this genus the size of the germ cells does not determine the size of the adult (Popoff, Chambers).

Within the same species differences in body size are due in the main to differences in cell number, the cell size being approximately constant. Small individuals are as complete and perfect as large ones, all the typical differentiations and organs being present in the former the same as in the latter. But in parts which are reduplicated, such as the gill filaments, lobules of liver, kidney, ovary and testis, etc., these parts are more numerous in large individuals than in small ones. In the parts which are reduplicated, whether they be organs or cells, there is practically no differentiation between the different members. Increase in size is due to an increase in the number of these parts or cells, without any increase in the total number of the various kinds of differentiations.

On the other hand, differential cell divisions, such as are found in the early cleavages of the egg do not vary in number in eggs or embryos of different size. The study of the cell lineage of these gasteropods shows that the cleavage is cell for cell the same in eggs and blastomeres of all sizes and species until ectomeres, entomeres and mesomeres are completely separated, and until differential divisions have given place to non-differential ones. So far as cell division is associated with differentiation and morphogenesis in the cleavage period, the number and character of these divisions do not vary in different species or individuals; so far as it is associated with growth, and the 'vegetative duplication of parts,' but not with differentiation, it may vary enormously. Pro-

fessor Whitman's ('93) statement that, "The organism dominates cell formation, using for the same purpose one, several or many cells," is true within certain limits. But the differences in cell number which are unimportant are only those which are associated with growth and not with differentiation, with trophic as contrasted with morphogenetic processes.

Supplementary Note. After this paper had gone to press I received an important publication by S. Morgulis entitled "Studies of Inanition in its Bearing upon the Problem of Growth," Arch. f. Entw.-Mech., Bd. 32, 1911. The author finds that both the size and number of cells are decreased in starving animals. Experiments of my own on starved planarians yield the same result.

LITERATURE CITED

- AMELUNG, E. 1893 Ueber mittlere Zellengrößen. Flora, Bd. 77.
- BEREZOWSKI, A. 1910 Studien ueber die Zellgrösse, I. Arch. f. Zellforschung, Bd. 5.
1911 Studien ueber die Zellgrösse, II. Idem. Bd. 7.
- BOVERI, TH. 1904 Ergebnisse ueber die Konstitution der chromatischen Substanz des Zellkerns. Jena.
- CHAMBERS, ROBERT 1908 Einfluss der Eigrosse und der Temperatur auf das Wachstum und die Grösse des Frosches und dessen Zellen. Arch. f. mik. Anat., Bd. 72.
- COLTON, H. S. 1908 Some effects of environment on the growth of *Lymnaea columella*. Proc. Acad. Nat. Sci., Philadelphia.
- CONKLIN, E. G. 1896 Cell size and body size. Abstract of paper read before Amer. Morph. Society. Science, January 10.
1897 The embryology of *Crepidula*. Jour. Morph., 13.
1898 Environmental and sexual dimorphism in *Crepidula*. Proc. Acad. Nat. Sci. Philadelphia.
1912 Cell size and nuclear size. Jour. Exp. Zool., vol. 12.
- DAVENPORT, C. B. 1897 The rôle of water in growth. Proc. Boston Soc. Nat. Hist., vol. 28.
- DE VARIGNY 1892 Experimental evolution. Macmillan, London.
- DONALDSON, H. H. 1895 The growth of the brain. Scribners, New York.
- DRIESCH, H. 1898 Von der Beendigung morphogener Elementarprozesse. Arch. f. Entw. Mech., Bd. 6.
1900 Die isolierten Blastomeren des Echinidenkeimes. Idem., Bd. 10.
- GATES, R. R. 1909 The stature and chromosomes of *Oenothera gigas*, DeVries. Arch. f. Zellforschung, Bd. 3.

- *GAULE, J. 1889 The number and distribution of the medullated fibers in the spinal cord of the frog. *Abh. d. Math.-physiol. Cl. d. konigl. Sachs. Gesell. d. Wissenschaft.*
- HARDESTY, I. 1902 Observations on the medulla spinalis of the elephant, etc. *Jour. Comp. Neur.*, vol. 12.
- JENNINGS, H. S. 1908 Heredity, variation and evolution in the Protozoa. *Proc. Amer. Philos. Soc.*, vol. 47.
 1909 Heredity and variation in the simplest organisms. *Amer. Naturalist*, vol. 43.
 1911 Assortative mating, variability and inheritance of size, in the conjugation of *Paramecium*. *Jour. Exp. Zool.*, vol. 11.
- JENNINGS, H. S. and HARGITT, G. T. 1910 Characteristics of the diverse races of *paramecium*. *Jour. Morphology*, vol. 21.
- LEVI, G. 1905 Vergleichende Untersuchungen ueber die Grösse der Zellen. *Verh. anat. Ges.*, Bd. 19.
 1905 Studi sulla grandezza della cellule. *Arch. ital. anat. embriol.*, T. 5.
- MINOT, C. S. 1908 Age, growth and death. Putnams, New York.
- MONTGOMERY, T. H., Jr. 1906 The oviposition, cocooning and hatching of an Aranead, *Theridium tepidariorum*. *Biol. Bull.* vol. 12.
- MORGAN, T. H. 1895 Studies of the 'partial' larvae of *Sphaerechinus*. *Arch. f. Entw. Mech.*, Bd. 2.
 1896 The number of cells in larvae from isolated blastomeres of *Amphioxus*. *Idem*, Bd. 3.
 1904 Relation between normal and abnormal development of the embryo of the Frog. III. *Idem*. Bd. 18.
- ORTON, J. H. 1909 On the occurrence of protandric hermaphroditism in the mollusc *Crepidula fornicata*. *Proc. Royal Soc.*, vol. 81.
- PEEBLES, FLORENCE 1897 Experimental studies on *Hydra*. *Arch. f. Entw. Mech.*, Bd. 5.
- POPOFF, M. 1908 Experimentelle Zellstudien. *Arch. f. Zellforschung*, Bd. 1.
- RABL, C. 1899 Ueber den Bau und die Entwicklung der Linse, III. *Zeit. wiss. Zool.*, Bd. 47.
- SACHS, J. 1893 Physiologische Notizen, VI. Ueber einige Beziehungen der specifischen Grösse der Pflanzen zu ihrer Organization. *Flora*, Bd. 77.
- SEMPER, C. 1876 Animal life as affected by the conditions of existence. Appleton's, New York.
- STRASBURGER, E. 1893 Ueber die Wirkungssphäre der Kerne und die Zellgrösse. *Histolog. Beiträge*, Bd. 5.
- WHITMAN, C. O. 1893 The inadequacy of the cell theory of development. *Jour. Morph.*, vol. 8.
- ZUR STRASSEN, O. 1898 Ueber die Riesenbildung bei *Ascaris*-Eiern. *Arch. f. Entw.-mech.* Bd. 7.

* Quoted from Donaldson ('95).

ON THE STRUCTURE OF CLINOSTOMUM MARGINATUM, A TREMATODE PARASITE OF THE FROG, BASS AND HERON

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SEVENTEEN FIGURES

PREFATORY NOTE

In a previous article an account was given of the distribution in this country and Canada of *Clinostomum marginatum* together with some notes on its habits. A short time after the publication of that article (Osborn, '11) Professor Linton informed me that he had recently found specimens of *Clinostomum marginatum* in brook trout which were taken from Alder Lake, a private preserve in the Catskill Mountains in New York. The conditions under which the trout live are well described by Linton ('10). "It is a lake of forty acres in the heart of the mountains. The owner maintains a well equipped hatchery on the stream below the outlet and allows no other fish than trout in the lake." It is thus clear that the infection takes place in the lake, or, in other words, that the first stages of this worm and its primary host are to be found there. The lake is visited by fish-eating birds and thus we can readily account for the introduction of the parasite. As pointed out in my previous article, we possess no information as to the early stages in the life history of *Clinostomum*. We do not know its first host nor anything about its development. It is evident from the facts now known as to the occurrence of the parasite at Alder Lake that the infection must come from some form living in that lake, very likely some invertebrate serving as food to the trout. Occurrence in a small lake narrows down the problem of discovering this missing

chapter in the life history of our subject to very workable limits. More favorable conditions for a study of the point could hardly be imagined. Professor Linton's communication also adds a new host and a new locality to our knowledge of the distribution of this animal.

The present paper gives an account of the organization of *Clinostomum marginatum*. In justification of this when two accounts are already extant I may plead the fact that neither of them are fully adequate and in some points both are erroneous. *Clinostomum* is a parasite of some of our most desirable game and food fishes and it is especially obnoxious because it is lodged in the edible portion of its host. In order to keep the paper within reasonable size I have left out many histological items and it is hoped that later, when certain points have received additional study, a further account of the histology may be published.

EXTERNAL FORM AND DIMENSIONS

The outer form of this, as of most trematodes, is extremely changeable. It has therefore seemed best to give a description of the form and measurements of worms after fixation. There is little difference in form and proportions of body between the late immature stages from cysts in the fish and frog and mature worms from the heron. The encysted worms appear to average very little smaller. Figs. 1 to 4 enable one to obtain an idea of the form of the animal. Fig. 1 is from a worm killed under compression, which, after carmine staining, has been mounted entire. Figs. 2 and 4 are from horizontal and transverse series; they show parts which are on different planes as if they were on the same level and need to be checked by the transverse sections shown in fig. 3. Fig. 3 shows transsections from seven levels, all drawn to the same scale. They are from a series of about one thousand sections and the accompanying number is that of the section in the series, and shows, though only very roughly, the distance of the sections from each other.

The body is subdivided into two regions separated at the level of the ventral sucker. Anteriorly it is almost cylindrical, its cross section being an ellipse, posteriorly it broadens considerably

and is frequently somewhat concave on the ventral surface. In living animals the posterior region of the body at times becomes momentarily flattened and may thin out to a sharp lateral fin but this is a merely momentary form followed by the thicker form seen in the sections. The constriction of the body at the level of the ventral sucker is shown in Wright's figure ('79, fig. 1) and in that of Linton ('98, pl. 44, fig. 6). It is also found in the other species of the genus as can be seen in several of the forms figured by Braun ('00).

The dimensions of thirty-nine individuals were obtained from worms mounted whole in balsam and drawn in outline with the camera lucida. The measurements were taken both from mature heron material and from bass worms.

These figures correspond fairly with those previously published except that the minimum one for length is the least thus far recorded. A part of the differences can doubtless be attributed

TABLE 1

Showing the length and width in millimetres of thirty-nine individuals of C. marginatum

FROM BASS		FROM HERON		FROM HERON	
FIXED IN COLD SATURATED AQUEOUS CORROSIVE		FIXED IN CORROSIVE		FIXED IN CHROMIC ACID (0.3 PER CENT)	
Length	Width	Length	Width	Length	Width
3.1	1.2	3.0	0.9	4.3	1.1
3.5	1.3	3.0	1.0	5.5	1.5
3.8	1.5	3.5	1.0	6.0	1.5
4.0	1.6	3.7	1.0	6.5	1.5
4.0	1.6	4.0	0.7	6.5	2.0
4.0	1.6	4.0	0.9	7.0	1.7
4.0	1.6	4.0	1.0	7.0	1.7
4.1	1.6	4.1	1.0	7.2	1.7
4.5	1.5	4.5	1.0	7.5	1.8
5.0	1.3	5.0	1.2	7.7	1.7
5.5	1.5	5.0	1.2	7.9	1.8
		5.2	1.2	8.2	1.9
		5.5	1.7		
		6.0	2.2		
		6.5	1.5		
		6.5	1.7		

to the great mobility of the animal, but the series is too regular to be wholly due to mere differences of degree of extension of animals of a constant length and indicates also the existence here of a length variation such as is common in all animal groups. They furnish further an indication that the encysted worms, which are slightly younger, are smaller than the heron worms. The average length of the eleven specimens from the bass is only 4.1 mm. while that of the sixteen worms from the heron, fixed with the same reagent, is 4.59 mm. It is interesting to note the larger figures for the chromic acid material. The average length of these individuals is 6.77 mm.

The form of the anterior end of the body is remarkable. In many distomes the walls of the body converge anteriorly and meet at the mouth, here they run parallel until they intersect the margin of a peculiar area, the oral field, which closes the anterior end of the body. In an animal in which the oral field is in the resting position, as in fig. 4, it is oblique to the axis of the body, with the dorsal side projecting somewhat beyond the ventral. It is this obliquity to which Leidy's generic name alludes. Fig. 1 shows the margin of the oral field where it meets the side wall. Often there is a slight depression in the margin of the field at this point. In fig. 5 the field is retracted, a very frequent act of the living animal; this section is from a specimen which was caught by the fixing reagent in this act. A fuller account of the oral field structure will be given later.

The ventral sucker (figs. 1 *w*, 3 *C*, and 4) is a very conspicuous organ, both in the whole animal and in sections and is much larger than the oral sucker. In all living and preserved animals which I have seen it is entirely enclosed within the contour of the body. Its opening is always very distinctly visible and is usually triangular, with one of the equal sides anterior and the apex posterior. In some cases, however, as in fig. 1, the three angles are rounded, or the opening (as in Linton, '98, pl., 44, fig. 6) may be circular or even almost square (Wright, '79). The sucker has a length of 0.7 mm. and the same width and measures 0.4 mm. dorsoventrally. It is about half as thick as the body and reduces its space very much, as shown in fig. 4 *C*. The reason for the

great size of the ventral sucker has not been indicated by the behavior of the animal. I have not seen it used at any time. Its histological structure is such that it would seem to be fully functional and its great size indicates a function of considerable importance but no activities have been observed in connection with it.

POSITION OF THE GENITAL PORE

MacCallum ('99, p. 699) states that "at about the middle portion of the body behind the ventral sucker the genital *openings* [*italics mine*] are seen, close together, that of the female apparatus being directly in front of the male." Also on page 703 he says that the female genital opening is located "directly in front of the male genital pore." These statements certainly imply that there are *two genital pores*, a condition not found elsewhere in trematodes. The statements are however contrary to fact and are not consistent with MacCallum's figs. 3 and 7 where a single genital pore is clearly shown, so that it is difficult to see how they crept into his paper. The exact position of the genital pore was determined for twenty-two individuals, the data for which are shown in table 2.

The total length and width/measurements are given and the distance from the anterior end to the genital pore. In order to make direct comparisons possible the position of the pore in percentage of total length is given in the column on the right. The opening is thus shown to lie posterior to the center of the body in every instance and to vary between 53.7 per cent and 68.3 per cent as extreme limits. A part of this difference may perhaps be attributable to individual differences of contraction or reagent action but in addition to these we must attribute it in part to variation in the actual position of the pore. If we take the average of these figures we should have 56 per cent as the point of location. The fact that some of the worms of this table show a greater length than any given in table 1 is because they are specimens killed under compression and are consequently unnaturally elongate. I have however admitted them to this table, as the

TABLE 2

Measurements used in determining the position of the genital opening

CATALOGUE NUMBER OF SPECIMEN	TOTAL LENGTH OF SPECIMEN	GREATEST WIDTH OF SPECIMEN	DISTANCE FROM ANTERIOR END TO GENITAL OPENING	PERCENTAGE OF TOTAL LENGTH TO GENITAL PORE
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	
b.....	3.8	1.2	2.5	65.7
x.....	4.4	1.3	2.8	63.6
l.....	6.0	1.5	3.4	56.6
750 b.....	6.0	2.3	3.4	56.6
n.....	6.2	1.6	3.8	61.2
750 e.....	6.7	2.3	3.6	53.7
734.....	6.8	1.75	4.1	68.3
g.....	6.9	1.7	3.8	55.0
e.....	6.9	1.75	3.8	55.0
d.....	6.9	2.0	3.8	55.0
h.....	7.0	1.8	3.8	55.0
a.....	7.2	1.8	4.2	58.3
762.....	7.5	1.8	4.2	56.0
750.1.2.....	7.5	2.0	4.25	56.6
f.....	7.5	2.3	4.3	57.3
762.....	7.75	1.75	4.5	58.0
k.....	7.8	1.9	4.5	57.7
i.....	7.9	1.75	4.6	58.2
m.....	8.3	2.1	4.9	59.0
c.....	8.8	2.1	5.3	60.2
750.1.....	9.2	2.3	5.1	55.4
750.a.....	10.8	3.0	6.1	56.4

compression may be supposed to have acted equally in all directions and so has not influenced these results.

A comparison of the figures of the different species of this genus as given in Braun's paper ('00) shows that the position of the genital pore differs very much in them, it being very near the posterior end of the body in *C. heluans* (fig. 10 *a*) 80.3 per cent, and very posterior also in *C. dimorphum*. It is nearly 79 per cent in the maximum case of *C. marginatum* of the table just given.

The genital pore opens into a chamber, the atrium (fig. 3) in which the male and female genital systems end. The opening of the terminal part of the uterus, the 'metraterm,' lies in this atrium anterior to the position of the cirrus of the male system.

This fact corresponds with the statements of MacCallum except in so far as he gives the impression that these openings are located on the outer surface of the body. The excretory pore opens (as shown in fig. 4) dorsally very near the posterior end of the body.

THE STRUCTURE OF THE BODY WALL

In general the trematode body is encased in a wall made up of a non-cellular cuticula, which may or may not be spinous, resting upon an outer zone of the parenchyma in which muscles run in various directions. For convenience we may consider the oblique muscles as marking the inner boundary of the wall though there is no break in histological structure at that point. The fibers of the oblique muscles lie in groups considerably spaced from each other so that the central parenchyma passes up between the muscles to the cuticle. This well-known structure is shown by Braun in *Fasciola hepatica* ('93, pl. 29, figs. 1, 2 and 3); it is also found in *Cotylaspis* (Osborn, '04, fig. 21) and in many other forms.

In *Clinostomum marginatum* there is a decided departure from the usual type which, since a similar structure has not been reported for any other trematode so far as known, merits a detailed description. Figs. 3, 4 and 5 show the relation of the wall to the body as a whole. The wall seems to be distinctly marked off from the central substance in these figures, due to the prominence of the large oblique muscles.

The cuticle is as usual. It measures from 0.01 to 0.015 mm. in thickness, is entirely structureless, is reinforced by spines which ordinarily do not project beyond the surface. The spines are acute and taper from a broader base seated on the deeper surface of the cuticle. They are set close together. Twin spines of smaller size sometimes occupy the position of one spine of ordinary size, as if the amount of embryonic material apportioned to one spine had been subdivided between two. Spines are found in all parts of the general surface of the body, they are more numerous on the ventral surface and on the posterior parts of the dorsal surface. They are not found generally on the surface of the oral field, with the exception of a small area immediately adjoining

the mouth opening. The spines have a strong affinity for stains and in the iron-haematoxylin preparations are deeply tinged by it, while the cuticle remains unaffected. We know nothing of the process by which spines are formed.

The principal peculiarity of the wall of *Clinostomum* is the existence in its inner layer of an extensive system of cavities, an extension of deeper cavities pervading the parenchyma everywhere, connected ultimately with the excretory collecting vessel. A full description of these cavities will be given in connection with the excretory system of which it forms a part. They are conspicuous in longitudinal sections and can be seen in fig. 4 *G* and figs. 6 and 7. The subcuticular cavities run in such a direction as to encircle the body, with connections inward to the collecting vessel as seen in transverse sections.

Organs in the cuticle, perhaps sensory. Certain cavities in the cuticle (see fig. 8) are possibly parts of sensory organs. They can be found in the areas in the oral field immediately around the mouth and on the dorsal surface near the anterior end, but not in the surface generally. In fig. 8 two of these are shown. They are spherical cavities excavated in the substance of the cuticle by which they are entirely enclosed except at the base of where they are open to the parenchyma on which the cuticle rests. The cavities thus have no communication whatever with the exterior. A fine deeply stained fiber can be traced into these cavities from the parenchyma. The indication from views like that of the cavity on the right in the figure is that this thread expands into a disk resting against the upper surface of the cavity. The best interpretation of the function of these organs which we can make on the basis of their structure is that they are the terminations of a pressure sense apparatus, the fiber being regarded as a prolongation from a more deeply seated nerve cell.

We have very few references to such organs in the cuticle of other trematodes. They are doubtless not uncommon but not many forms have been examined for them. Nickerson ('95) found a very similar organ in *Stichocotyle*, one of the *Aspidobothridae*. The organ shown in his fig. 15 differs only in size from the one in fig. 8 of this article. Bettendorf also ('97, fig.

30) found organs of much the same kind in the oral sucker of certain distomes. Pratt ('09, fig. 10) copies a figure of a section of the body wall of *Ligula*, one of the cestoda, from Zerneke. The section made by the Golgi method shows nerve cells located some distance below the cuticle from which threads run outward to small spherical 'sense organs' located in the basal level of the cuticle. These organs and those of Nickerson are similar in structure to those of *Clinostomum*. In *Cotylaspis* (Osborn, '04, fig. 33) the cuticle contains organs apparently of sensation but of a different type from these. They are in the surface of the cuticle and communicate with the exterior. They have a number of stainable fibers which unite and pass as a single thread inward through the cuticle and disappear in the parenchyma. Nickerson, in the article just referred to, in his fig. 14 has shown an organ in the cuticle which communicates with the exterior.

Muscles of body wall. The usual muscles are present as in trematodes at large. Figs. 6 and 7 show them in longitudinal and transverse section respectively. In addition to them there is a layer of longitudinal muscle, which lies immediately below the cuticle. This is an unusual layer of longitudinal muscle, the usual one being located inside the circular muscle, while this is external to it. We may designate it the outer longitudinal muscle (*mo*) the other being then called inner (*mi*) in the figures. The fibers of this outer longitudinal layer were seen by Looss ('85) and are shown in his fig. 23. According to his figure they are very much stronger than I find them in my sections. In my material the fibers are exceedingly small, having a diameter of only 0.0009 mm. In fig. 7 they are shown under a magnification of 1100 diameters. Their size can perhaps be better appreciated by a comparison with those of the inner longitudinal layer as seen in figs. 6 and 7. In the latter the fibers are cut transversely. These fibers lie at equal distances apart, in a single layer, and in direct contact with the cuticle.

Writers who have given attention to the finer structure of trematodes (Braun, '93; Otto, '96; Stafford, '96, to mention three at random) agree that there are three layers which compose the musculature of the body wall, viz: circular, longitudinal and

oblique. I have recently made a re-examination of the sections on which my paper of 1904 was based to test the possibility of the coat being present in *Cotylaspis*; as a result I am entirely convinced that there is no outer longitudinal muscular layer. It thus seems safe to conclude that *Clinostomum* is peculiar in the possession of this layer, though a similar may perhaps be found later in some other forms. My observations of the other coats also confirm those already reported by Looss. The fibers of the circular coat lie in several layers (fig. 7); they are very small, though larger than those of the outer layer, measuring 0.0012 mm. They do not fall into groups or bundles like those of the inner longitudinal layer. These fibers are seen in sections generally at various levels between the sub-cuticular excretory cavities, which thus seem to occupy an area produced by the expansion of that part of the body wall, in correlation with the presence of these cavities.

The inner longitudinal muscles lie much deeper than usual. Instead of lying quite near the cuticle as they do in many cases, and in close contact with the circular muscles, they are located here, as shown in fig. 6, below the vessels of the excretory system at a distance of 0.04 mm. from the outer muscles. The inner muscles are thus seen to be pushed down to a considerable depth below the bottom of the cuticle near which they usually lie. This departure from the ordinary arrangement is clearly a structural adaptation correlated with the presence of the sub-cuticular vessels. We may further perhaps regard the presence of the outer longitudinal muscles as a part of the same adaptation; they may have been developed thus near the surface to offset the disadvantage due to the increased distance of the inner longitudinal layer from the cuticle.

The inner longitudinal muscle fibers are very distinctly grouped into bundles alternating with intermediate areas from which they are absent. Fig. 7, *mi*, shows one of these bundles in cross section; it is made up of a cluster of fibers without other muscles in close proximity. The fibers of the inner longitudinal muscle differ in size, as can be seen in fig. 7; the largest ones are much larger than those of the circular muscle, measuring 0.004 in diam-

eter. The oblique muscles running in the usual two directions are more deeply located.

Certain interesting points were noted in the cytology of the body wall muscles which will receive attention later in connection with those of the parenchyma.

Wall structure in the oral field. The wall of the oral field presents a structure decidedly unlike that of the general surface of the body. Figs. 3 and 5 show the wall under low magnification. It is very much thinner, owing to the great reduction of all its components. The cuticle becomes so thin as to be barely recognizable. The spines, which are so general over the rest of the surface of the animal, are entirely wanting on the oral field with the exception of a small area immediately around the mouth opening where spines of a much smaller size exist. Sub-cuticular cavities so conspicuous elsewhere are scarcely recognizable. They do not in any case take on the regular arrangement so usual elsewhere in the body wall, but are merely irregular cavities underlying the surface and communicating internally with the vessels of the excretory system. The musculature of the oral field does not agree with that of the rest of the body. The various layers are not continued from the wall into the field. Fibers can be found lying parallel with the surface but they cannot be connected with the fibers in the wall beyond. The longitudinal muscles of the parenchyma (*pml* in fig. 3) run on anteriorly until they meet the surface of the field to which they are then vertical. They are shown in fig. 5 at *ml* running directly to the wall, their position enabling them to act as retractors of the field as shown in the figure.

Glands (?) in the body wall. There are certain nucleated cells lying in the body wall, as shown in fig. 7 at *gl*, which seem to be probably of a glandular nature. They are very long and slender, consisting of a globular body, which lies on the level of the oblique wall muscles, and a tapering portion which can be traced outward to a termination on the inner surface of the cuticle. The outer end of the cell may branch so as to present in sections two terminations. No passage through the cuticle has been seen or any indications of secretions passing from these cells to or through it.

The globular body of the cell is entire on its inner side; that is, there are no processes given off from it. The bodies of these cells contain a large clear nucleus. There is no cell wall. The cells stain readily with iron-haematoxylin. Their bodies which lie on nearly the same level constitute a faint zone parallel with the surface of the body.

The position and, to a certain extent the structure of these cells, remind one of the cells found by Blochmann ('96) in trematodes and cestodes in a similar situation. I have not had access to this paper of Blochmann, but several writers have reproduced his figures, among them Pratt ('09) who, in his recent paper on the cuticula, copies a figure from Blochmann of the wall of the cestode *Ligula* and designates 'sub-cuticular cells' certain cells which show great resemblance to those of *Clinostomum* to which I have just referred. There are some differences between the cells in Pratt's figure and those in fig. 7 of this article. In *Ligula* the cell body is sharply angulated on its inner side and produced into fine threads, which are lost in the deeper parenchyma. Externally also the cells soon taper to a very fine thread. In spite of these differences however it seems quite reasonable to regard these cells in their relations to the body structure as a whole as identical with the sub-cuticular cells of *Clinostomum* just described. Benham ('01) gives a diagram of the structure of the body wall of *Ligula* which has cells more like those seen in *Clinostomum*. It is held by some writers that these are epithelial cells which have sunken from a position originally on the surface. The *Clinostomum* sections do not supply any evidence in support of the view that these cells are epithelial in origin.

THE PARENCHYMA

The interspaces among the organs within the body are permeated by the usual network of branching fibers emanating from large nucleated cells. In places where the parenchyma comes in contact with the surface of the walls of various organs such as the oesophagus (fig. 8) and the uterus, but not of all (not of the intestine, for example) its fibers become much more numerous

and denser, so as to form a compact capsule for the support of the parts beneath.

There are certain cells loosely clustered together in a mass which lies in the parenchyma in the region directly anterior to the ventral sucker. They are shown in figs. 1, 3, 4 *B* and 5. These cells are oval, and measure 0.03 by 0.015 mm. The nucleus, which lies near the margin of the cell, is clear and round and contains one or more nucleoli and a few minute grains of chromatin. These cells lie among those of the parenchyma but differ from them in appearance, having no processes and no connection with the fibers of the parenchyma. Each cell is sharply bounded. They also have no connection with the surface, no processes can be traced from them going off toward the surface and their long axes lie in all directions. If they communicated with the surface the cell bodies would point in that direction. There seems to be no doubt that they are purely internal in their physiological action. They are similar in cytological appearance in both bass and heron worms. The cells contain a clear homogeneous material which has a marked affinity for stains.

The physiological significance of this organ is entirely unknown. Looss recognized these cells in the immature worms from the fish cysts. He suggests ('85, p. 46 of separate) "vielleicht sind es die Anlagen von Drüsen, die später. . . . erste ihre Funktion antreten werden." But this suggestion cannot be accepted since the cells are identical in structure in the mature worms. MacCallum's suggestion ('99, p. 700) that they are parenchyma cells cannot be accepted, for they are not found outside of certain limits and parenchyma cells pervade all parts of the body. The great number of these cells leads one to believe that they are important. Their entire absence of connection with other organs implies an independent function. It seems therefore most likely that they are concerned in some way in internal secretion.

Parenchymal muscles. The muscles of the parenchyma are very well developed in Clinostomum. The usual two sets are found, (fig. 3) namely, the longitudinal muscles and the dorso-ventral ones. There are no horizontal muscles. As the longi-

tudinal muscles pass forward they ultimately meet the oral field almost vertically to its surface and attach there so that they thus become its retractors. Fig. 5 is a camera drawing from a specimen which died with the oral field retracted. In this section the longitudinal parenchyma muscles can be traced forward directly to the in-bent parts of the field. Observations of sagittal sections furnish evidence that, at least in many cases, a single muscle reaches across from the dorsal to the ventral surface, for nucleated myoblasts can be seen in connection with these muscles and these are grouped in the center of the body.

Some cytological features are well shown in the muscles of *Clinostomum*. Both the inner longitudinal wall muscles and the longitudinal parenchyma muscles frequently show transverse subdivisions into stained and unstained zones such as has been noted in other forms by various writers on trematode histology, but in no case with which I am familiar are they shown so distinctly as here. Nickerson ('95) states that in *Stichocotyle* the longitudinal muscles of the body wall appear to be tubular "with nodes of deeply staining substance filling the lumen at intervals," and shows the appearance in fig. 16 of his paper. Stafford, too, in *Aspidogaster* ('96, fig. 26) noted the presence of 'transverse lineations' in the parenchyma muscles which he speaks of as 'contraction centers.' He does not note any in connection with the body wall muscles. Bugge ('02) in his paper on the excretory system in cestodes and trematodes incidentally mentions 'Querstreifung der Muskelfasern' which he observed in the circular and longitudinal muscles of redias and cercarias, "wie wir sie bei Arthropoden und andern Wirbellosen auffinden," and also quotes Cerfontaine, to whose article (in Bull. Acad. Sci. Belg., 27, no. 6) I have not had access, and Nickerson as having seen the same thing. In 1904 I saw and recorded ('94, figs. 11 and 12) a similar muscular structure in *Cotylaspis*, a form related to *Stichocotyle* and *Aspidogaster*.

Turning now to *Clinostomum*, figs. 9, 10 and 11 are from immersion objective camera drawings of longitudinal wall and parenchyma muscles. Figs. 9 and 11 are from the body wall and parenchyma muscles respectively from the same series. Both

were drawn with the same objective but 11 is made with a higher eyepiece. Such views are found generally in many different series so that we are justified in regarding them as a normal feature of the cytological structure of Clinostomum muscle. In fig. 11 it is clearly seen that the muscle is made up of several parallel-sided filaments of considerable length, composed of a substance which is not strongly influenced by haematoxylin and a second deeply staining substance. The swollen globular appearance of the latter leads us to believe that it is a peculiar 'contractile substance.' The fibers do not all present this appearance. One is represented in fig. 10 in which also the myoblast and nucleus are shown. The myoblast is large, measuring 0.014 mm. across, and the nucleus has a diameter of 0.005 mm. Fibers can be traced from such myoblasts. These appear differently from those in figs. 9 and 11, showing a dark contour on the wall and a clearer center. In cross sections the appearance is that of two substances, a clearer central and a darker surface material. These seem to be the 'hollow muscles' of writers. Fig. 10 shows the fibers, probably in an uncontracted state, while 9 and 11 are contracted fibers. A more adequate study of the cytology of this muscle is beyond the scope of this article.

THE ALIMENTARY APPARATUS

Oral sucker. The mouth opening lies in the center of the oral field and leads into the cavity of the oral sucker. The sucker is nearly spherical and is very much smaller than the ventral sucker, measuring 0.28 mm. long and 0.25 mm. across. It has the usual cuticular lining and heavy muscular wall composed of fibers running in the various directions.

Oesophagus. The pharynx, which is generally present in trematodes and usually follows close after the oral sucker, is entirely wanting. There is a short tube immediately behind the oral sucker which, after running ventrally a short distance, makes a dorsal bend to meet a transverse portion of the intestine. This is the oesophagus. The structure of the two bends is somewhat different. The more anterior portion is very thin walled and is lined with a thin cuticle continuous with that of the oral sucker.

The posterior chamber is a globular dense body as seen in a whole-mounted worm. The wall is thick and heavy, due not to the presence of a heavy muscular coat as it would be if the organ were a pharynx, but to the very peculiar structure of the cuticular coat. The cuticle here, which is continuous with the thin layer of the anterior chamber, suddenly changes its character and becomes a mass of tall slender processes springing vertically from the wall and projecting freely into the lumen of the organ. Their appearance is shown in fig. 12. They bear some resemblance to the tall processes of the epithelium of the intestine just above them, with which they are directly continuous. They have every appearance of having arisen from a cuticularized epithelium. In *Cotylasspis* (Osborn, '04) there are similar indications of an epithelial origin of the cuticle which lines the oesophagus.

The posterior chamber of the oesophagus has almost no muscular tissue in its wall. A very few circular and longitudinal fibers can be recognized, evidently strictly comparable with the muscles of the intestine. There is however a coating on the inner surface of the organ which is a condensation of the parenchyma at large. This has a fine but definite boundary next the parenchyma.

Oesophageal glands. Numerous cells lie in close proximity to the oesophagus (*oegl* in fig. 12) which are not ordinary parenchyma cells. Their massing too goes to show that they constitute a definite organ whose position requires us to regard it in its work as in some way a part of the oesophagus. The cells are not angular like those of the parenchyma but have rounded outlines. Each cell has a nucleus poor in chromatin and a distinct nucleolus. The sharp line in the figure passing on the left side of this group of cells marks the boundary of the denser parenchyma which ensheathes the oesophagus. It will be seen that the cells are located outside of this sheath and so are somewhat remote from the lumen of the oesophagus. An organ of this sort is usual in trematodes; it is often called a 'salivary gland.' One writer however, (Otto, '96) questions the salivary function of the cells in the Amphistomes, since he does not find any connection between them and the lumen of the oesophagus. We shall however go on calling these organs 'oesophageal glands' though we have no definite

information as to their physiological significance. It is possible that they are merely mucin-forming organs and that they acquire a temporary connection with the oesophagus.

The intestine. The intestine consists of a part crossing the body transversely (fig. 4 A) which, after bending, continues as the two long lateral caeca. The caeca lie in the center of each half of the body and extend (fig. 2) to the level of the excretory bladder. The walls of the caeca are not entire; blind pouches extend outward from them. These pouches are not as large and distinct in the material after fixation as they are in life. Fig. 13 a is a free-hand drawing of the living organ in a specimen just liberated from a bass cyst. The pouches arise on both sides of the intestine; they are very numerous and close together and are not long and slender. The form of these pouches distinguishes *C. marginatum* from *C. heterostomum*. In the latter (Braun, '00, fig. 1) there are a few very long and slender diverticula which are confined to the outer side of the intestine. In the presence of these intestinal pouches *Clinostomum* resembles *Fasciola hepatica* and the planarians. Fig. 15 is a camera drawing of the wall of the intestine. The pouches are conspicuous in some places and absent in others. This corresponds with the facts seen in life; in bass specimens the pouches are contractile and at moments are drawn back into the wall. The wall itself is contractile; in life its movements are very conspicuous. The lumen is filled with a fine grained material lemon yellow in color. This flows back and forward, streaming, the pouches empty themselves of it or fill with it and the contractions of the wall may obliterate the intestine entirely for a moment.

The structure of the intestine wall from a fully matured heron worm is shown in fig. 13. The epithelium presents two distinct zones, a deeper basal one and, arising from it, a second zone of separate columnar structures. The basal zone is a continuous protoplasmic layer, in which distinct nuclei occur at somewhat regular intervals, but without any walls dividing it into cells. This basal syncytium takes the stain readily. The processes of the outer zone show a relation to the nuclei below though they are not always strictly over them. In a section, (fig. 13 on the

left) this may be due to slight differences in level. It is planned to treat certain cytological points connected with this epithelium in a later paper so that for the present I will only state that these processes are apparently amoeboid and capable of being projected from the deeper body of the cell or retracted. They are clear and barely stained. They are filled with minute black pigment grains which have been traced to the decomposed blood corpuscles of the heron on which the worm had fed. While the intestinal epithelium in some instances shows the appearances just described there are other cases in which its form is quite different as shown in fig. 14. Here it is a low, level surface consisting of a layer of protoplasm with imbedded nuclei. There are no division walls and the layer has the appearance of a syncytium. Cells of the type shown in fig. 15 are found in the intestine of worms from the bass. I have interpreted them as being in a resting or non-digestive state while those in fig. 13 are actively engaged in the work of digestion. In many trematode sections and figures with which I am familiar the cells of the epithelium are entirely distinct and independent to their very base. They do not show any fusion as if syncytial as is the case here. In connection with the structure of *Cotylaspis* ('04, fig. 19) I called attention to the entire independence of the cells of the intestinal epithelium. In *Cryptogonimus*, on the other hand, the cells of the epithelium of the intestinal caeca are fused into a syncytium.

The circular muscular coat is very scanty, its fibers lie close to the epithelium. The longitudinal coat is also very feeble. Its fibers lie at a distance in the parenchyma.

The cavity of the intestine of the worms obtained from bass cysts is filled with thin, flat, four-sided crystalline bodies. As soon as the worm has escaped from its cyst the strong peristaltic contractions already mentioned force this substance backward and forward. At frequent intervals portions of it are expelled from the body through the mouth. In worms obtained from the heron this material is not found in the cavities of the intestine. In such worms on the contrary the intestine has been found to contain a coagulated fluid substance with blood corpuscles scattered through it, which upon careful examination were found to be

identical with blood corpuscles taken from the heron. This substance is evidently food, but the content of the caeca of the bass worm cannot be so considered. Its prompt rejection from the body as soon as it is liberated from the cyst would be evidence sufficient to justify this conclusion. Its crystalline form and the fact that it is discharged as soon as the animal becomes free, point to the hypothesis that the cavities of the intestine are made use of for storage during encystment and that the substance therein is a waste product.

THE EXCRETORY SYSTEM

The excretory system of *C. marginatum* has never been described. The indications of it which are given in Looss ('85, fig. 22) are purely diagrammatic and somewhat misleading. The system, moreover, presents some features which are very unusual, so that the whole subject needs a careful revision.

The location of the excretory pore has already been noted. It opens from a very short duct (fig. 15) which is in communication with the v-shaped bladder. Internally the two branches of the bladder receive the termination of the collecting tube in the center of a flattened area. At the excretory pore there is an invagination of the cuticle which covers the outer surface of the body. As this passes more deeply it gradually changes into a cubical epithelium composed of nucleated cells identical in structure with those which make the wall of the collecting vessel. At intermediate points epithelial cells of the bladder show all stages of degeneration in structure and pass insensibly into cuticle. There is no muscular tissue in the walls of the bladder. Living specimens were observed particularly with reference to contractions in the bladder as I had found this organ in *Cotylaspis* interesting in this respect ('94, p. 216) but the walls were not contractile. In correlation with this is the absence of a sphincter at the surface pore (one is present in this place in *Cotylaspis*) and the presence of a sphincter at the junction of the collecting vessel and the bladder. We may conclude from the position of the sphincter and the non-contractility of the bladder that the latter in *Clinostomum* is

merely a passage and not a place of storage and that the collecting vessel is the functional bladder.

The collecting vessels are very large in the posterior region of the body, but anteriorly their identity is lost. It is usual in trematodes for a collecting vessel to run from the terminal bladders forward to a point near the anterior end of the body and then to bend suddenly on itself and run backward again. The second vessel, called the recurrent vessel, is supplied with a strong vibratile apparatus, while the collecting vessel lacks these. In *Clinostomum* the collecting vessel is readily traced forward as far as the ventral sucker. In fig. 2 it is shown on the right side omitted on the left, in fig. 3 it is shown as far forward as the genital organs and then omitted, in fig. 4 it is shown in sections *B-G*. It disappears a short distance in front of section number 150. The level of the vessel is seen from the cross-sections. It always lies externally to the caeca and generally slightly ventrally. Its diameter is quite variable as in fig. 15, a camera drawing of a section passing in its plane for a long distance. The wall is epithelial and muscular.

I am not able to give a definite account of the relation between the collecting vessel and the recurrent vessel. I have devoted much time to the study of this in different ways without being able to follow the collecting vessel forward to where it meets the recurrent vessel. The body is too thick anteriorly to allow this point to be seen in an entire compressed specimen. I have repeatedly examined the youngest individuals I could find but without success. The network of anastomosing vessels (described in a moment) are so complicated in the anterior of the body that it is impossible to recognize the collecting vessel if, indeed, it has remained distinct from them. It is, of course, possible that the collecting vessel does not remain distinct but is lost in the network of vessels.

Allusion was made above to the system of cavities which lie in the body wall immediately under the cuticle. In living worms just removed from bass cysts these cavities are filled with a cream-colored fluid composed of minute highly refractive droplets which has the effect of an injection, making it very easy

to distinguish the different vessels and their connections. In such a preparation the collecting vessel as well as the smaller vessels which are derived from it are readily seen. The appearance of this system of vessels is shown in a free hand drawing (text fig. 1, for which I am indebted to Mr. Faus Silvermale) made from an unusually young live bass worm under slight compression. The collecting vessel can be followed, its size diminishing as it advances until it is lost in the network of its subdivisions. The network shows a predominance of transverse vessels, though

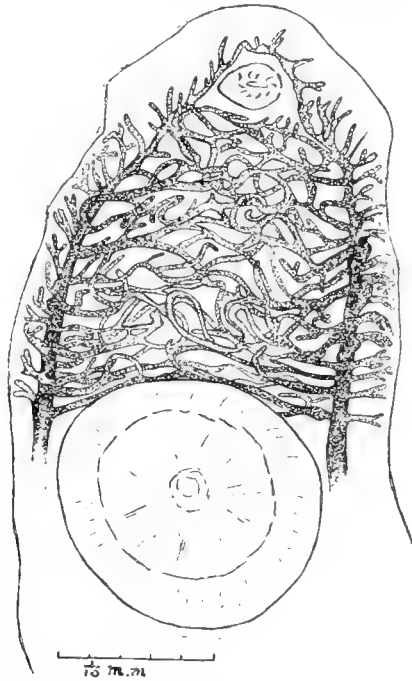


Fig. 1 Free hand drawing from a young, living, slightly compressed bass worm. Zeiss 2 A.

with many communicating vessels connecting them, and some which cross over to the other side of the body and become continuous with those of that side. In this preparation the recurrent vessel could not be seen, but as I shall point out later it seems most likely that it is present and joins the collecting vessel in this part of the animal.

In the posterior part of the body these vessels may be somewhat more definitely subdivided into two sorts according to their destination, a superficial system which runs to the surface and becomes the subcuticular vessels and a deep system which passes

inwardly among the inner organs. The origins of both kinds can be seen in fig. 15; they arise at close intervals from the collecting vessel on both sides. Some of the vessels of the superficial system run directly to the surface (one of these is shown in fig. 4 G) where they become circular vessels immediately under the cuticle. Since these tubes encircle the body they are readily seen in sections passing tangentially in the plane of the surface. The tubes frequently anastomose and all communicate directly with the collecting vessel. They are cut across in longitudinal sections and produce the appearance seen in figs. 3 and 5.

Looss ('85, p. 49) expressed a suspicion that these subcuticular vessels communicate directly with the exterior: "so halte ich es doch für höchst wahrscheinlich, dass—der Excretionsporus nicht die einzige Stelle ist von welcher dieses Maschenwerk von Kanälen mit der Aussenwelt in Verbindung tritt und zwar sind es die subcuticularen Maschen des Gefässnetzes welche diese Kommunikationen vermitteln." And later he says "Ich halte es nun nicht für unmöglich dass sie (i.e., subcuticular vessels) auch nach aussen münden," etc. But he closes his account with an admission to the effect that he has not succeeded in demonstrating the presence of openings from them to the exterior. The observations of the movements of the fluids in these passages described above render it very certain that outlets from them directly to the exterior do not exist; were such outlets present we should undoubtedly have seen stuff from within issuing through them.

Turning now from these superficial vessels to the deep ones we find that they pass inward, permeating the parenchyma everywhere. The vessels of one side tend to remain entirely confined to that side, they anastomose with one another but do not often become continuous with those of the opposite half of the body.

Allusion has been made to the flow of the contents of the excretory vessels in life. Pulsations were seen in bass specimens in the wall of the collecting vessel, forcing a stream out into the dependent vessels. Later these streams reversed their direction and the droplets course back again into the larger vessel. As already noted there is no escape distally; the movement is an ebb and flow.

An observation made recently upon a worm from a frog cyst seemed to be inconsistent with a circulatory movement of the droplets as just described. The cyst attracted my attention because of its small size, it being globular, compact and only 1.3 mm. in diameter, quite unlike frog cysts and much like those found in the bass. When the worm had been liberated and arranged in a compressor for observation it was seen that the vessels of the anterior region were filled with highly refractive droplets. These droplets were not in a state of flux but were stationary. The pulsations mentioned above and the flow of droplets were seen in bass specimens in the posterior region. In the frog worm the vessels of the posterior region were empty. It is possible that this worm was a very recent arrival in the frog and that the movements of the droplets had not yet begun to take place.

A system of branches derived from the collecting vessel and permeating the body in this way is very unusual in trematodes. The usual structure is a network of minor vessels uniting to form larger vessels which finally merge into a single collecting vessel. In three widely separated forms however we find an arrangement somewhat similar to that of *Clinostomum*. In a young stage of *D. echinatum* Looss ('04, fig. 192) figures a collecting vessel much resembling that of *Clinostomum*, especially in the anterior body region where side branches are given off, the main vessel meanwhile continuing until it meets the ciliated recurrent vessel at the extreme anterior end of the body. A comparison of Looss' fig. 192 with that of the younger stage shown in 191 indicates that the branching is a late feature in the life history, a fact of interest since it is uncommon in trematodes at large. In adults of *D. echinatum* (Looss, '94, fig. 114) these vessels are very much branched but the branches do not assume the form of a sub-cutaneous system like the one so well developed in *Clinostomum*. In *Cephalogonimus* also the excretory collecting vessel is branched. This point was first noticed by Poirier ('85, fig. copied by Braun, '93, pl. 20, fig. 9) who says "Ces canaux lateraux comme le canal impair median, emittant sur tout leur parcours des branches ramifiees se dirigeant vers le bords lateraux du corps. Ces ramifications s'entendent en avant, jusque un peu au-dela du point de bifurcation de l'oesoph-

age." In his illustration, as well as in this description, there is no recognition of subcuticular vessels like those of *Clinostomum*. In a study of the parasites infesting the frogs of Minnesota I have happened to find specimens of this genus. The study of living and sectioned material of this form demonstrates that, while there is an extensive system of vessels derived by branching from the collecting vessels and one which bears considerable resemblance to that found in *Clinostomum*, encircling subcuticular vessels are not developed.

A third form with somewhat similar branching excretory vessels was encountered at Chautauqua, New York. In the livers of sun-fishes certain cysts were found which contained immature flukes belonging to the holostomes. These forms are peculiar in having a broad thin anterior body region bearing a resemblance to the foot of a gasteropod mollusc and posteriorly a globular mass carried vertically over it. The excretory pore is located at the summit of the latter. In the thin anterior part there are a median and two lateral longitudinal vessels, extending from which are branching vessels extending everywhere in the foot, anastomosing and forming a complete network. All of the vessels of this system contained minute highly refractive droplets, similar in appearance to those found in the excretory cavities of *Clinostoma* which had been recently liberated from bass cysts. In the living worms masses composed of these droplets were discharged from time to time from a point located at the posterior end of the body, the excretory pore, thus indicating that the passages are members of the excretory system. In life the droplets were in constant motion in the vessels, coursing rapidly in all directions as they had been seen doing in *Clinostomum*.

This observation, taken in connection with the presence of such droplets in the encysted specimens of *Clinostomum* and their absence in the heron specimens of *Clinostomum* and the free living *Cephalogonimus*, constitutes an argument in favor of the supposition that the droplets are composed of chemical wastes. In an encysted organism these must be disposed of in a way that will prevent their damaging the animal, accordingly they cannot be discharged from the body in the ordinary manner but must be

stored during the period of encystment. It is thus reasonable to look upon the extensive equipment of spaces possessed by the excretory collecting vessel as a storage apparatus. In favor of this interpretation is the further fact that in both *Clinostomum* and the holostome just mentioned the contents of these cavities begin to be discharged as soon as the worm has escaped from its cyst. I think that the substances contained in the intestinal caeca may also prove to be waste matters and that these cavities are also being employed for storage.

The recurrent excretory vessel. Reference has already been made to a vessel which parallels the collecting vessel. It is readily seen in the parts of the body behind the ventral sucker; anteriorly it is lost in the maze of vessels which are derived from the collecting vessel. Posteriorly (fig. 16) it bends sharply forward, as the vessel into which the capillaries drain. This, which I have called the recurrent vessel, is spirally coiled in all sections and even shows this state in living animals. It is located externally and somewhat dorsally to the collecting vessel, but is much smaller, having a diameter of only 0.02 mm. The wall is composed of a very thin membrane. The tube is uniform in diameter in all parts; unlike the collecting vessel the wall possesses no contractility, there being no muscular tissue present. The wall of this vessel is supplied at close intervals with peculiar ciliary organs. In life these vibrate at a very rapid rate so that they become visible only after their vitality has become lowered. Then it is seen that the organ is attached posteriorly in the wall of the vessel, the rest being free and pointing anteriorly so that its vibration produces a current running forward in the tube. These organs are located in the recurrent vessel at close intervals. Bugge ('02, fig. 62) finds that in certain cercarias occurring in certain helices the chief canals are supplied with 'Wimperschopfen' which correspond with the organs just mentioned and in addition that there is a lining of ordinary cilia clothing the rest of the inner surface of the wall. There are no similar ordinary cilia in these vessels of *Clinostomum*. The ciliary organs are many times longer than the diameter of the vessel in whose lumen they lie. In life I am unable to recognize individual cilia in them but in sec-

tions after the application of iron-haematoxylin, cilia are clearly seen as sharp black wiry looking lines.

In view of the fact that these ciliary organs produce strong current which flows forward, we are compelled to suppose that the recurrent vessel discharges directly into the collecting vessel, although as already noted it has not been possible to recognize the connection.

Flame-cells and capillaries. The ultimate members of the excretory apparatus of Clinostomum are very imperfectly known as yet. Much attention and time have been dedicated to the effort to trace these parts in the living animal with very inadequate reward. Some glimpses of them have been obtained however both in life and in sectioned material. Flame-cells have been seen; they are very tall and slender with a narrow base where the elongate and narrow mass of cilia are attached. A detailed account, with illustration of these flame-cells together with some other finer points, must be reserved for a later article.

It has not been possible to determine the mode of arrangement of the capillaries and connecting vessels. In some places the capillaries have been recognized. They ran in a posterior direction. Vibrating ciliary organs could be seen within them. It was not possible in any case to trace these vessels to a point of connection with the recurrent vessel and I feel very strongly convinced that the recurrent vessel does not receive any branches.

EXCRETORY SYSTEM IN TREMATODES IN GENERAL

There is considerable difference in the plan of anatomical organization of the excretory systems of different trematodes. In all there is a system of flame-cells and their capillaries and one, or occasionally two (e.g., *Aspidogaster*), posteriorly located excretory pores. But there is great difference as to the vessels lying between the external pore and the capillaries. All degrees of distance between the terminal bladder and the capillaries can be found. In *Opisthoglyphe endolobum* (Looss, '94, fig. 157) a large forked chamber, confined to the posterior third of the body, receives directly a vessel formed by the junction of the capil-

laries. In *Allocreadium isoporum* (Looss, '94, fig. 15) a collecting vessel can be recognized which reaches the first body third and there receives the capillary vessels. In *Gorgodera cygnoides* (Looss, '94, fig. 125) a collecting vessel runs the whole length of the body and at its anterior end meets a vessel which runs backward a short distance before the connecting vessel from the capillaries meets it. This might be considered as a short recurrent vessel. In *Distomum echinatum* (Looss, '94, fig. 191) a fully developed collecting vessel meets a still longer recurrent vessel. In *Harmostomum leptostomum* (Looss, '94, fig. 113) the collecting vessel is fully developed and the recurrent vessel runs nearly to the posterior end before the two vessels enter it from the capillaries. Finally in *Cotylaspis* (Osborn, '04, fig. 26) the recurrent vessel as well as the collecting vessel, is fully developed, the capillaries discharging into a canal which is a forward bend of the recurrent vessel. We see from this brief survey of these different forms that, with the gradual development of both collecting and recurrent vessels, an increasing interval is interposed between the capillaries and the exterior. The structure of these two vessels is entirely different, one being entirely destitute of ciliary apparatus and furnished with muscular tissue, the other being ciliated and devoid of muscle. Clinostomum has its place among the forms with complete collecting and recurrent vessels, and in addition possesses the remarkable system of branches derived from the collecting vessel.

It would be possible to find a series of forms showing reciprocal developments of excretory collecting vessel and bladder. Thus in *Stichocotyle* there is no bladder and the collecting vessels are very large; in the closely related *Cotylaspis* the collecting vessels are narrow tubes and there are two well developed excretory bladders which are rhythmically pulsatile.

While noting that the parts of the excretory system thus exhibit a series of degrees in the development of complexity of organization we must not forget that this series is found not in a group of genetically related animals but among forms which are widely separated in the system. On the other hand when we examine forms which are closely related we find great differences. Thus

in the three genera of the Aspidobothridae we find in *Stichocotyle* (Nickerson, '95, fig. 23) no recurrent vessel, a very voluminous collecting vessel and no bladder; in *Aspidogaster* (Stafford, '96, fig. 15) a partly developed recurrent vessel, moderate collecting vessel, no bladder and two excretory pores; in *Cotylaspis* (Osborn, '94, figs. 5 and 26) a fully developed recurrent vessel, a small collecting vessel and two well developed bladders. So that it is impossible to attach any phylogenetic value to differences shown in the organization of the excretory system.

THE REPRODUCTIVE SYSTEM

My observations are in substantial accord with those of Looss and MacCallum with regard to the chief facts in the anatomy of this system. Figs. 1, 2 and 3 show the parts in situ as they appear in various sectional planes. Fig. 17 is an outline of the organs based largely on a study of the total preparation from which fig. 1 was drawn. The two testes lie in the last body third; ovary, shell-gland and ootype, oviduct, Laurer's canal and yolk receptacle are all compactly grouped in the space between the testes. There is a peculiar uterine sack in the course of the uterus, the yolk follicles are small and very diffuse. Since no detailed account of the members of this system has ever been given I will give here a brief description of it.

Genital pore. The genital pore lies in the mid-ventral line (fig. 4 *E*). Its distance from the anterior end has been considered already in this article. There is an atrial cavity below the surface from which a single pore opens to the exterior. The relation of these parts is shown in figs. 3 and 4 *E*.

Cirrus sack. MacCallum's figure shows the cirrus sack and its contents very adequately. The sack occupies a position on the right side of the animal in front of the anterior testis. At its posterior border it receives the two vasa deferentia. The wall of the sack is supplied with muscles whose powerful fibers lie so as to form a circular and longitudinal coat. The sack contains a tube of varied diameter, coiled so as to accommodate its length to that of the sack. Posteriorly the tube expands to a larger, thin-walled, non-muscular seminal vesicle filled with spermatozoa.

Continuing it anteriorly is a smaller portion whose wall is supplied with a very strong coat of circular muscles. It is followed by a less muscular portion at the outer end of the sack. This portion is surrounded by what are apparently to be regarded as prostate cells. This part is not as strongly muscular as the middle region of the tube. Following the usual nomenclature I have designated the middle and outer parts respectively as prostate portion and ductus ejaculatorius. It seems however that the more glandular part and the more muscular parts are out of the usual order. Thus in *D. isoporum* (Looss, '94, fig. 104), the outer part is strongly muscular, and coiled and between it and the seminal vesicle there is a small chamber with which the large prostate cells communicate.

Testes. The testes are somewhat pyramidal in shape, their bases slightly concaved and facing each other. In some cases the remaining surfaces are more or less deeply indented, in many others they are entire. A sharp line bounds the testes. The vasa deferentia have a wall of epithelium with flattened nuclei. This epithelium can be recognized in the wall of the testis where it connects with the vas deferens but no epithelium can be seen in the wall of the organ elsewhere. Apparently trematodes differ on this point. In *Cotylaspis* (Osborn, '94, fig. 88) the wall of the organ is distinctly epithelial. Schwartz ('85) found nuclei in the wall of the testis of certain early stages of trematodes while Ziegler ('83) claimed that the wall is non-cellular in *Bucephalus* and *Gasterostomum*.

The testes are filled with cells which, in some bass worms, are almost completely filled by the very large nucleus, poor in chromatin, and with a very large, readily staining nucleolus which indicate the inactive stage preceding spermatogenesis; in other bass specimens with cells showing various phases of spermatogenesis. In the heron worms the testes contain fully developed spermatozoa scattered among the active cells.

Ovary. The size and form of the ovary as shown in fig. 26 of Looss' paper ('85) is unlike anything which I have found in my material, in which it is oval, entire and measures 0.4 mm. by 0.2 mm. In MacCallum's figure it is also small and entire. It is

bounded by a thin non-cellular membrane, which encloses cells, some of which, near the opening to the oviduct, are much larger than the rest and are about to descend to the ootype.

Oviduct and uterus. The oviduct passes inwardly from the ovary and crosses to the opposite side of the body. Its wall is composed of cubical epithelium and circular muscle fibers. At a point near the ovary there is a small sack, the spermatic receptacle, opening from the oviduct, this narrows dorsally to a tube—Laurer's canal—runs to the dorsal surface of the body (fig. 4 *G*), and opens to the exterior. The epithelium of the oviducal wall is replaced by cuticle in Laurer's canal, which becomes continuous with that of the general surface of the body. The oviduct, at a point a little farther to the left, meets the duct coming from the yolk receptacle. There is no marked change in the diameter of the oviduct at this point but it is surrounded by glandular cells and doubtless serves as the ootype. The duct from this point continues as the uterus, at first without change in diameter or direction, next with several loops it recrosses toward the ovary, then abruptly bends again and runs a straight course, passes externally to the anterior testis on its left side and runs forward to enter a large sack which we may call the uterine sack. The relation of the uterus to this sack is shown in fig. 3; it passes on its dorsal wall for a distance and opens into it at about the center of its dorsal surface.

Uterine sack. The uterine sack is a large cavity capable of considerable distension; in the case of mature worms it is filled with eggs, as in fig. 1; in bass worms (fig. 2) the cavity is merely a narrow slit. The form of the cavity in transverse section is shown in fig. 4 *D*. It extends posteriorly to a point near the anterior border of the anterior testis; anteriorly it does not reach the ventral sucker. The outlet from the sack is located at its posterior end. The histological structure of the wall of the sack is quite unlike that of the uterus. In the latter there is a nucleated epithelium and a coat of muscle fibers. In the sack the cavity is lined with cuticle and there is a muscular coat consisting of circular and longitudinal fibers. In addition to these there is a condensation of the parenchyma immediately surrounding the uterus. The nuclei of these parenchyma cells lie in definite

lines parallel with the surface from which the fibers of the parenchyma radiate. A sharp line bounds this mass of specialized parenchyma which thus constitutes a capsule enclosing the uterine sack. The fact that the uterus enters the sack in the center of its dorsal surface and not at the anterior end prevents us from regarding the sack as merely a dilatation of the uterus. We must however think of it as having arisen as a differentiation which has taken place in a loop of the uterus. In most of the species of this genus (Braun, '00) there is a similar blind sack into which the uterus enters and which extends blindly in front of the end of the uterus. In one species however, (*C. heterostomum*, Braun, '00, fig. 1), the uterus passes forward to the posterior border of the ventral sucker where it bends and runs straight back again to end at the female genital opening. This is doubtless the more primitive anatomical arrangement, and the one from which the sack form has been developed. We note also in passing that this species is more primitive, too, in possessing well developed diverticula of the intestinal caeca.

The form of the uterine sack in the *D. reticulatum* of Looss, as described and figured in his paper ('85), is decidedly different from that which I have just described. The sack in that species is elongated posteriorly to reach a point posterior to the posterior testis (fig. 22). In fig. 26 we learn that the part of the sack which leads to the exterior is a lateral offset from the main sack. This posterior portion of the sack of Looss is wholly wanting in my material. Cross sections (e.g., fig. 4 *F*) show that the sack does not extend into the testis region of the body. This is an interesting point. It does not seem possible to doubt the fact as related by Looss for his form. In every other respect the form *D. reticulatum* bears the closest resemblance in organization to *C. marginatum*, and writers from Leuckart down have considered them identical. Thus Leuckart ('89, p. 401) says *D. reticulatum* "Mit Leidy's *Clinostomum gracile* zusammenfällt." Stiles and Hassall ('98) say "Looss described as *Distomum reticulatum* a form which is evidently identical with Leidy's *Clinostomum* as Leuckart has already surmised," etc. And MacCallum ('99, p. 705). The description given by Looss of *D. reticulatum* applies so exactly in every particular to the forms we

have just considered [*C. marginatum*] that I have not the least hesitation in concluding that they are the same." The form of the uterine sack however is very different in *D. reticulum* from that of *C. marginatum* or of any other member of the genus. According to fig. 26 of Looss' article ('85) the sack is extended posteriorly dorsally to the testes until it reaches a point posterior to the posterior testis. This posterior development of the sack is a feature not found in any of the species of this genus so far as I am aware. In the several species included by Braun ('00) in his article on the group, the sack ends in advance of the genital pore. If Looss was not in error in regard to the form of the sack (and this seems very improbable) then we must recognize that *D. reticulatum* differs decidedly in this respect from the rest of the genus. However in any case we should not attach very much importance to differences in the shape of an organ like the uterus or its parts.

Metratrum. There is a short slender tube running from the sack to the atrium (figs. 3 and 4 *E*) which, following the nomenclature suggested by Ward ('94), may be called the metratrum.

Vitellaria. The vitellaria are shown in fig. 1. They are diffusely scattered in all parts of the region behind the ventral sucker. As shown by transverse sections they lie in a thin zone, concentric with the surface and next to the outer wall of the body. They are entirely absent from the anterior part of the body. The vitellaria are made up of ultimate follicles, all of them very small and numerous measuring 0.07 mm. These are bounded by a distinct membrane which encloses a few yolk cells which measure 0.0125 mm. in diameter. Usually the vitellaria cannot be seen in total preparations made from bass worms, but sections of similar immature individuals show the follicles with their thin wall enclosed cells whose structure is then identical in appearance with that of the immature germinal cells of the testes and ovary. In sections from mature worms the follicles contain similar immature cells and also fully formed yolk cells with large nucleus and nucleolus and a cytoplasm containing granules, some of them measuring 0.001 mm. in diameter. These are food granules; in producing them the follicle cells differ from ovarian cells with which they are very likely homologous.

The yolk receptacle lies near the ovary and its duct reaches the oviduct as already noted. The shell gland surrounds the ootype at this point. Its cells radiate from the ootype and their long tapering portions seem to communicate with its cavity, though it is not possible to recognize absolutely the manner of connection.

The egg. The eggs measure 0.099 mm. by 0.66 mm. There is a distinct operculum very near the end of the shell. The shell in some cases is deeply stained by the haematoxylin and looks as if composed of the same substance as the spines. In other cases the shell is not influenced by the stain. The shells contain as usual a fertilized cell derived from the ovary and several cells derived from the vitellaria. The eggs, both those of the uterus and the older ones of the uterine sack are practically undeveloped. In some cases the true egg cells may undergo one or more of the early stages of cleavage but in the vast majority of eggs no development takes place during the time that they are lodged within the body of the parent. In view of this we must recognize the sack as merely a place for the storage of eggs. The reason for this storage remains for the present unknown.

BIBLIOGRAPHY

- BENHAM, W. B. 1901 A treatise on zoology, edited by E. R. Lankester. Vol. 4. The Platyhelminthes.
- BETTENDORFER, H. 1897 Ueber Musculature und Sinneszellen der Trematoden. Zool. Jhrb., Abt. Anat., Bd. 10, pp. 307-385.
- BLOCHMANN, F. 1896 Die Epithelfrage bei Cestoden und Trematoden. Hamburg.
- BRAUN, M. 1893 Bronn, Klassen u. Ordnungen; Platyhelminthes I, Trematoden. Bd. 4, pp. 396-924.
- 1900 Die Arten der Gattung Clinostomum Leidy, Zool. Jhrb., Abt. f. Syst., Bd. 19.
- BUGGE, G. 1902 Zur Kenntniss des Excretionsgefäßsystems der Cestoden und Trematoden. Zool. Jhrb., Anat., Bd. 16.
- LEUCKART, R. 1889 Die Parasiten der Menschen, u.s.w.
- LINTON, E. R. 1898 Notes on the trematode parasites of fishes. Proc. U. S. Nat. Mus., vol. 10 pp. 507-548.
- 1910 The diagnosis of a case of parasitism in the brook trout. Proc. Seventh Internat. Zool. Congress, Boston Meeting, 1907.

- LOOSS, A. 1885 Beiträge zur Kenntniss der Trematoden, *Distomum palliatum* n.s. und *D. reticulatum* n.s. Zeit. f. w. Zool., Bd. 41.
- 1894 Die Distomen unserer Fische und Frosche. Bibliotheca Zoologica. Abt. 16.
- 1899 Weitere Beiträge z. Kenntniss der Trematoden Fauna Aegyptens. Zool. Jhrb., Abt. f. Syst., Bd 12. pp. 521-784.
- MACCALLUM, W. G. 1899 On the species *Clinostomum heterostomum*. Jour. Morph., vol. 15, pp. 697-710.
- NICKERSON, W. S. 1895 On *Stichocotyle nephropis*, a parasite of the American lobster. Zool. Jhrb. Abth. Anat., Bd. 8.
- OSBORN, H. L. 1904 On the habits and structure of *Cotylaspis insignis* Leidy. Zool. Jhrb. Abt. f. Anat., Bd. 21, pp. 201-242.
- 1910 On the structure of *Cryptogonimus chyli*, etc. Jour. Exp. Zool., vol. 9, pp. 517-536.
- 1911 On the distribution and mode of occurrence of *Clinostomum marginatum*, etc. Biol. Bulletin, vol. 20, pp. 350-366.
- OTTO, RICHARD 1896 Beiträge. z. Anat. u. Histol. der Amphistomeen. Deutsche Zeit. f. Thiermedizin. u. verg. Pathol, vol. 22. (Inaug. Diss. Leipzig.)
- PRATT, H. S. 1909 The cuticula and subcuticula of Trematodes and Cestodes. Americ. Naturalist, vol. 43, pp. 705-729.
- POIRIER, J. 1885 Contrib. a l'histoire des Trematodes. Arch. Zool. Exp., ser. 2, vol. 5, p. 465.
- 1886 Trematodes nouveaux ou peu connus. Bull. de la Soc. Philom., ser 7, tom. 8.
- SALENSKY, W. 1874 Ueber d. Bau u. d. Entwk. der Amphilina. Zeit. f. w. Zool. Bd. 24, pp. 28-32.
- SCHWARTZ, W. 1886 Die Postemb. Entwk. der Trematoden. Zeit. f. w. Zool., Bd. 43.
- STAFFORD, J. 1896 Anatomical structure of *Aspidogaster conchicola*. Zool. Jhrb. Anat., Bd. 9.
- STILES AND HASSALL, 1898 Notes on parasites, no. 48. An inventory of the genera and sub-genera of the trematode family Fascioloidae. Archiv. f. Parasitology, vol. 1, pp. 81-99.
- WARD, H. B. 1901 On the structure of the copulatory organs in *Microphalus*. Univ. Nebraska. Zool. Studies, no. 43, pp. 175-187.
- WRIGHT. '79. Contributions to American Helminthology, I. Proc. Canadian Institute, vol. i.
- ZIEGLER, H. E. 1883 *Bucephalus* und *Gasterostomum*. Zeit. f. w. Zool., Bd. 39, pp. 537-571.

ABBREVIATIONS

<i>crs</i> , cirrus sack	<i>mt</i> , metraterm
<i>cu</i> , cuticle	<i>nc</i> , nerve collar
<i>dej</i> , ejaculatory duct	<i>nvs</i> , sensory nerve endings
<i>epo</i> , outer part of epithelium of intestine	<i>ocgl</i> , oesophageal glands
<i>epi</i> , inner portion of the same	<i>oes</i> , oesophagus
<i>exbl</i> , excretory bladder	<i>os</i> , oral sucker
<i>excv</i> , collecting vessel of excretory system	<i>otp</i> , ootype
<i>expo</i> , excretory pore	<i>ov</i> , ovary
<i>exrv</i> , recurrent vessel of excretory system	<i>pgl</i> , parenchyma glands
<i>gl</i> , glands communicating with the surface	<i>pi</i> , parenchyma sheath of wall of intestine
<i>gpo</i> , genital opening	<i>pn</i> , parenchyma cell nucleus
<i>int</i> , intestine	<i>prs</i> , prostate part of cirrus organ
<i>lc</i> , canal of Laurer	<i>ps</i> , parenchyma sheath of oesophagus
<i>mc</i> , circular muscle of body wall	<i>spn</i> , spines of body wall
<i>mi</i> , inner longitudinal muscle of body-wall	<i>ta</i> , anterior testis.
<i>mo</i> , outer longitudinal of the same	<i>tp</i> , posterior testis ~
<i>mob</i> , oblique muscles of body wall	<i>ut</i> , uterus
<i>mpl</i> , longitudinal muscles of the parenchyma	<i>utsk</i> , uterine sack
<i>mpt</i> , transverse muscle of the parenchyma	<i>vs</i> , ventral sucker
	<i>vsm</i> , seminal vesicle
	<i>vd</i> , vas deferens
	<i>vt</i> , vitellaria
	<i>vtd</i> , duct from vitellaria
	<i>vtr</i> , yolk receptacle

PLATE 1

EXPLANATION OF FIGURES

All the figures (except 10, 13 a, and 16) were drawn with the Abbe camera lucida. Most of them have been reduced one-third in reproduction; the magnifications are after this reduction.

1 View from the ventral surface *C. marginatum*, from a specimen from the throat of *Ardea herodias*, fixed under compression in aqueous corrosive sublimate, borax-carmine. $\times 12$.

2 A partly schematic view from the dorsal side, combining facts from several sections from a frontal series. From a bass worm. The vitellaria are not yet developed, the uterine sack is not dilated, the excretory collecting vessel is omitted from the right side and the recurrent vessel from the left, parts on different levels are shown on the same level. $\times 27$.



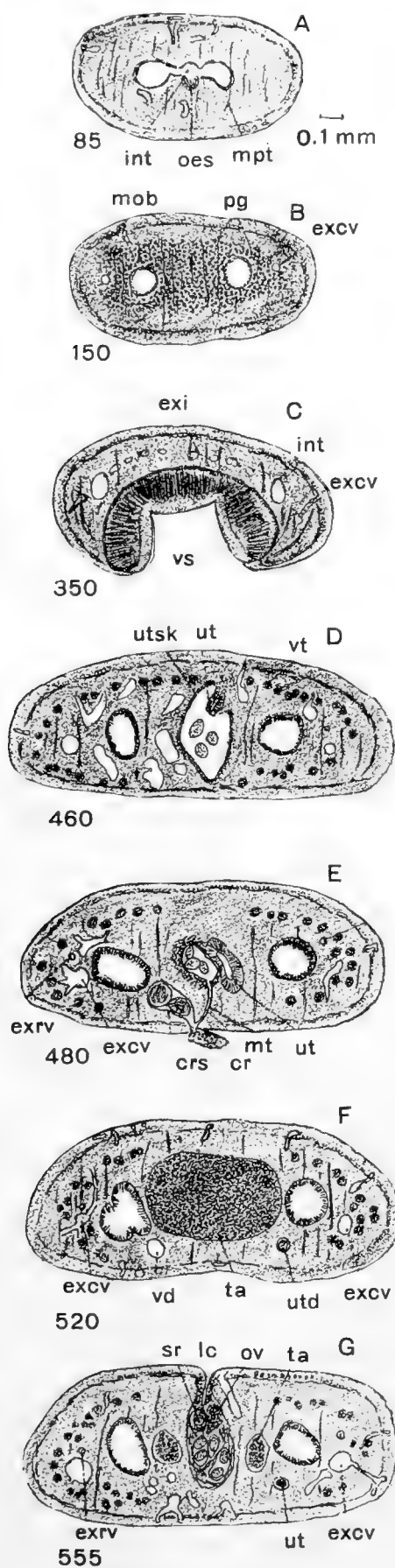
PLATE 2

EXPLANATION OF FIGURES

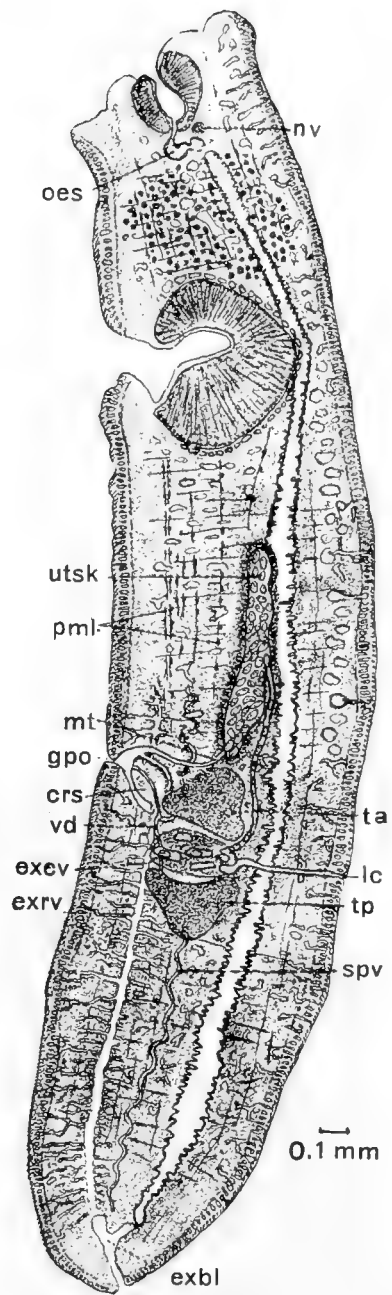
3 View combined from sections of a sagittal series, showing together organs which are on different levels, mouth, ventral sucker, genital organs and excretory pore are median while the intestine and the collecting and recurrent excretory vessels are lateral. $\times 27$.

4 Sections from a transverse series. The numbers show the number of the section in the series; *A* is in the level of the oesophagus; *B*, in front of the ventral sucker; *C*, at the ventral sucker; *D*, at the uterine sack; *E*, at the genital pore; *F*, at the anterior testis; *G*, at the canal of Laurer. From heron, corrosive and acetic, iron-haematoxylin. $\times 27$.

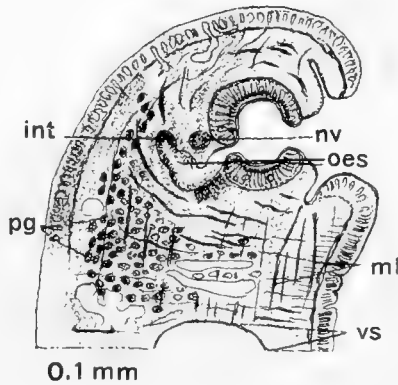
5 The center section of a sagittal series, from a worm which died with the oral field inverted. Heron, after chromic acid fixation and iron-haematoxylin. $\times 40$.



4



3



5

PLATE 3

EXPLANATION OF FIGURES

6 A longitudinal section of the body wall of the dorsal surface, showing the position of the various excretory vessels with reference to the muscular layers. Heron, corrosive, iron-haematoxylin. $\times 240$.

7 Body wall from a transverse section near the center of the ventral surface, showing the uni-cellular glands (?); chromic acid, iron-haematoxylin. $\times 1100$.

8 Two sense organs of the cuticle from the dorsal anterior region of the body. $\times 1100$.

9 Part of one of the fibers of the inner longitudinal muscle of the body wall, showing the alternation of stained and unstained substance. Heron, corrosive, iron-haematoxylin. $\times 560$.

10 Myoblast and its nucleus and adjoining muscle fibers of one of the longitudinal parenchyma muscles. Heron, chromic, iron-haematoxylin. $\times 1100$.

11 Part of one of the parenchyma muscles from the same series as fig. 9. $\times 1100$.

12 From a section passing vertically to the posterior region of the oesophagus. Heron, chromic. $\times 560$.

13 Section from a fully matured worm vertical to the wall of the intestine, showing the pseudopodial inner borders of the epithelium; the darker shading of the deeper ends of the cells indicates the distinction between the stained and little stained parts of the cell, corrosive, iron-haematoxylin. $\times 1100$.

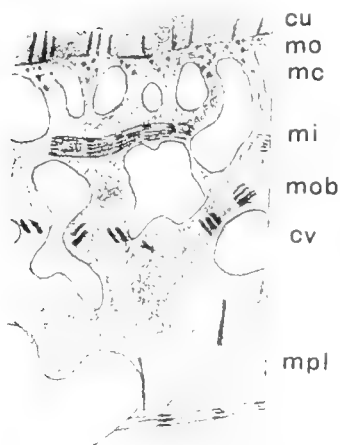
13 a Free hand drawing from a living worm from bass, showing the lateral pouches of the intestinal caeca.

14 The epithelium of the intestine from an immature worm showing resting stage of the tissue. Corrosive, iron-haematoxylin. $\times 1100$.

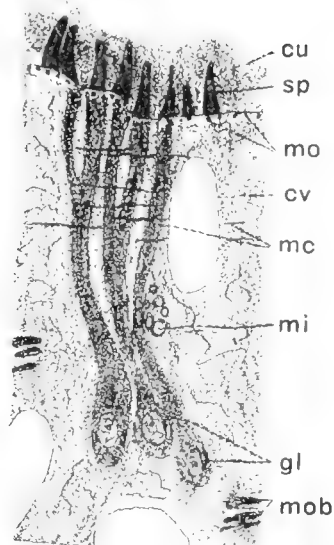
15 Reconstruction from several adjoining sections of a frontal series, showing the relation of the collecting to the subcuticular cavities and to the bladder, also the recurrent vessel and the intestine. $\times 36$.

16 Free hand drawing from the posterior ends of the chief excretory vessels as seen in a living worm from the bass under slight compression. \times Zeiss oc. 2, ob. A.

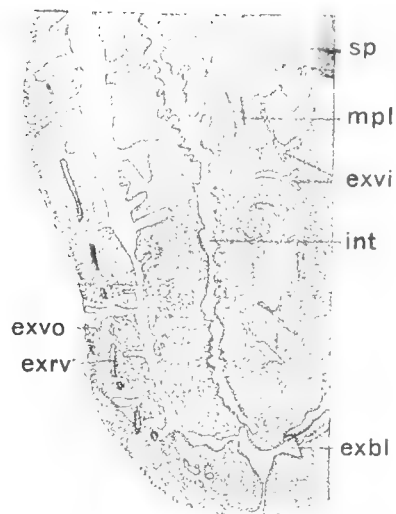
17 Reproductive system as seen from the ventral surface, from total preparations. The vitellaria have been omitted.



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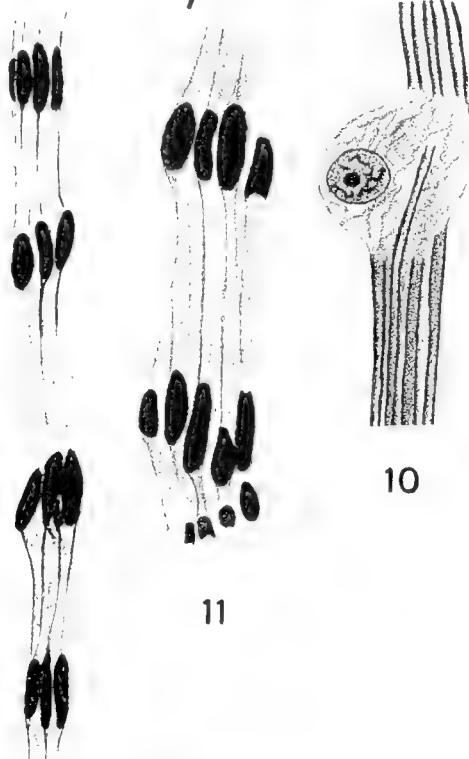
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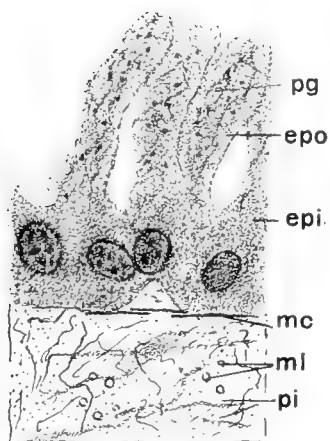
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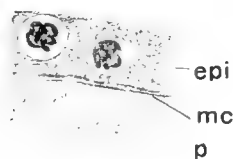
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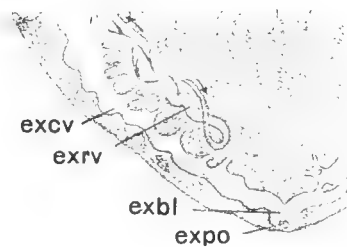
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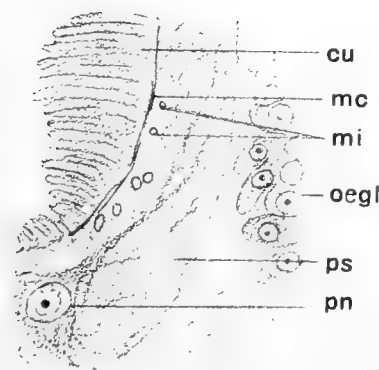
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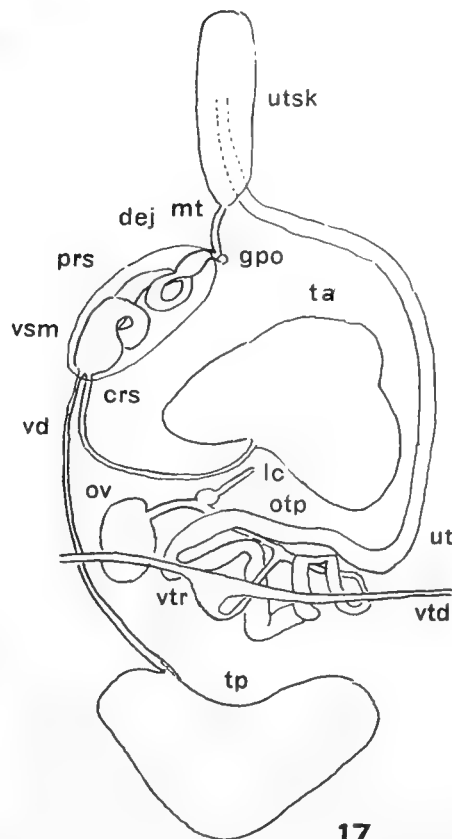
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13a



17

THE DEGENERATIONS IN THE SECONDARY SPERMATOGONIA OF DESMOGNATHUS FUSCA

B. F. KINGSBURY AND PAULINE E. HIRSH

From the Histological Laboratory, Cornell University

TWENTY-ONE FIGURES

In 1902 there was published a part of the results of a study of the spermatogenesis in *Desmognathus fusca* in which the occurrence of degenerations¹ in the secondary spermatogonia was mentioned. A fire had destroyed the larger portion of the preparations and photographs covering this portion of the spermatogenetic cycle, and no effort was then made to complete the investigation by a detailed study of the spermatogonia.

Of the degenerations that occurred two were particularly attractive—the regressive changes in the spent lobule and the degenerations in the last generation of spermatogonia, after the cessation of the season's transformation into spermatocytes. These degenerations are described as occurring in spermatogonia of the last generation, although they might quite as well, perhaps, as will appear subsequently, be given as involving spermatocytes at the very beginning of their growth period.

The occurrence of degenerations in the amphibian spermatogenesis has been known since the pioneer work of Flemming ('87) on the spermatogenesis of *Salamandra*. He described (p. 447) the vacuolation and fragmentation of the nucleus and the dissolution of the cell, constituting a form of degeneration already described by him ('85). He says:

Irgend eine Beziehung zur *Spermatogenese* kann diese Erscheinung keinesfalls haben, da sie zu einer viel früheren Zeit auftritt, als jene; in den Präparaten von Hoden mit Spermatogenese, welche ich bisher studiert habe, waren derartige Bilder nicht vorhanden, ich will jedoch

¹ Kingsbury, '02, p. 108, p. 111.

nicht behaupten, dass sie nicht auch gleichzeitig mit der Samenfadenbildung noch vorkommen könnten. Nach dem ganzen Habitus aber handelt es sich offenbar um Prozesse der *Degeneration* und des *Untergangs* von Kernen und Zellen, die aus einstweilen unbekannten Ursachen zur Zeit der Epithelwucherung in manchen Cysten eintreten, und die, wenn auch in der Form nicht ganz gleichartig, am nächsten vergleichbar erscheinen mit der *chromatolytischen Entartung* der Kerne im ovariellen Follikelepithel, die ich kürzlich an anderem Orte beschrieben habe (Flemming, '85).

A definite localization in the spermatogenetic cycle was thus not given, though it was recognized that it occurred much earlier than the period of 'spermatogenesis' at the time of cell proliferation. The cause was neither ascertained nor suggested.

Hermann ('81), in the same material of investigation, devoted (p. 99) more attention to these degenerations, describing the changes in the chromatin and achromatic substance in the nucleus, their altered staining reactions (as was subsequently done by Heidenhain and others). He noted the common occurrence of the degeneration in a certain kind of cell (his 'spermatocytes'), the degeneration of entire lobules and the freedom from degeneration of the follicle cells. He regarded the degeneration as normal and, in connection with the question of its significance, he commented on the extravagance in the outlay of germ cells, calling in as illustration the atresia folliculi.

Drüner ('93) devoted an article of several pages to the attempt to show that the degenerations in the testis of *Salamandra* described by Flemming and Hermann, were due to a parasite (protozoan, coccidian) whose spores entered the nucleus. His view will be commented on subsequently. Later workers on amphibian spermatogenesis (Meves, McGregor, Montgomery, and others) have not, so far as we are aware, noted the occurrence of such degenerations as have been described in *Salamandra*.

Whatever may be the condition in other salamanders, aside from the European form in which these degenerations have been recorded as given above, in *Desmognathus* studied by us they are of constant occurrence. The material collected and studied covers a period of ten or twelve years and is from localities in the vicinity of Ithaca one to two miles apart. The occurrence of the degenerations becomes striking when it is recognized that they

occupy in this form a distinct place in the spermatogenetic cycle. Their occurrence, in fact, is so closely associated with the annual spermatogenetic cycle and with the 'polarity' of the testis in *Desmognathus* that a brief description may be introduced, even at the expense of essential repetition of descriptions which have been previously published (Kingsbury, '02).

The spermatogenetic cycle in *Desmognathus* may be said to begin in the fall or late summer, after the extrusion from the testis of the spermatozoa formed during that season. During the fall and winter months there is a multiplication of the spermatogonia and a tardy growth of the spermatocytes I, which in midwinter seems practically suspended. Some spermatocytes undergo division but the maturation divisions appear to be often abnormal and the resulting cells to degenerate. In the spring the multiplication of the spermatogonia and the growth of the spermatocytes begins actively, characterizing particularly the months of March, April and May, while divisions of the spermatocytes occur in May, June and July. The transformation of the spermatids into spermatozoa preponderates in August and September.

In the late spring or early summer, transformation of the 'last generation' of spermatogonia stops, no more spermatocytes beginning their period of growth, until fall, and it is after this time, when the growth of new spermatocytes has ceased, that the degenerations in question occur. The cells undergoing degeneration have been spoken of as secondary spermatogonia of the last generation although, since they come intermediate between the spermatocytes I and the secondary spermatogonia, they might, perhaps, be equally as well designated as young spermatocytes I.

The following out of the sequence of stages in the spermatogenesis of *Desmognathus* is facilitated by the marked polarity of the organ in this form. This polarity seems to be rather characteristic of many at least of the tailed amphibia (Meves, McGregor, Kingsbury, etc.) and particularly perhaps of the smaller or more elongated ones. The changes of spermatogenesis proceed as a 'wave' in a cephalo-caudal direction so that at the proper season

of the year, mature or maturing spermatozoa fill the lobules at the cephalic end while primary spermatogonia occupy the caudal filament of the gland, successive stages occupying in orderly sequence the intervening groups of lobules. Compare Kingsbury ('02), text figure A.

In *Desmognathus*, in the spring and in some cases in early summer, the transition from spermatogonia to mature spermatocytes I, as shown in longitudinal section of the organ, is gradual, but as the season advances a 'boundary plane' between those destined to furnish spermatozoa that season and those that will hold over becomes more and more evident. The 'spermatogenetic wave' has stopped short at a particular point; the progressive changes of spermatogenesis continue in one portion and lag or are lacking in the other portion; and it is in those lobules just behind (caudad of) this boundary that the degenerations are constantly found.

How striking this boundary between the two regions becomes may be seen by comparing figures 1, 2, 6 and 3 which reproduce longitudinal sections of the testis of *Desmognathus* from the months of May, June, August and September respectively, the transverse lines drawn upon the photograph indicating the plane of boundary which in figure 1 is not yet established as such. Figures 4, 7, and 5 give enlarged views of the region of the boundary and in them the cysts and lobules filled with degenerating cells are seen. Figure 7 is from a section neighboring that shown in figure 4 which is an enlargement from figure 2, while figure 5 is an enlargement from the section of figure 3. As is well recognized in urodele spermatogenesis, the cells contained in and composing a cyst are in the same or closely contiguous stages of development, and while in some instances, cells occur singly or a few together, in the great majority of cases, the contents of the entire cyst or even lobule are undergoing degeneration together. In the degenerating cysts and lobules however it is the germ cells and not the follicle cells forming the cyst wall that degenerate, the nuclei of the latter being apparently normal and distinguishable amid the degenerating spermatogonia as Hermann pointed out and figured. Figures 8 and 9 show degenerating lobules while

figures 10, 11, 12, and 13 show cysts in different stages of degeneration. In most of these figures the nuclei of the follicle cells may be recognized in the midst of the degenerating germ cells, fragments and débris.

Although occurring constantly and abundantly in the summer months in this region of the testis, the degenerations appear sporadically at other seasons. They have been found in late fall (November) and in the spring (April). They are not as abundant at these seasons but occur in the same region, i.e., in the zone intermediate between the secondary spermatogonia and the growing spermatocytes. It is obviously difficult to determine whether such cells are spermatogonia or are young spermatocytes, or whether they are all destined to disintegrate or whether some may 'recover' from the extreme 'contracted' condition of the nucleus which is so characteristic of early stages in this form of degeneration. It was this that first suggested a comparison with synizesis. This, however, will be discussed subsequently.

The determination of the exact sequence of changes that occur in the cell is difficult as it involves the larger question of the structure and functional changes in the nucleus and cell body. No attempt has been made to follow systematically the changes in the cytoplasm which are elusive and attention has therefore been turned more particularly to the nucleus where the effect is striking.

Degeneration in the nucleus seems to set in when the cell is preparing for mitosis. In the prodromic stage the nuclei possess a clear appearance with a definite delicate reticulum. It should of course be appreciated that the point of immergence is inferred from the structure of the apparently unchanged nuclei in the same cyst or the same lobule. This inference may be seen to be justified if it be remembered that the cells in the same cyst are in nearly identical stages, the same applying, but less closely, to the lobule. Three such nuclei are shown in the upper left hand corner of figure 12. The definite changes of degeneration are initiated by the contraction (collapse) of the nucleus such as is shown in figure 10. There then appear in the degenerating nuclei numerous spherical masses often lying in clear spaces as if in vacuoles (figures 11 and

12). With iron hematoxylin they retain the stain strongly as do the nucleoli. The application of a more differential stain, such as the Biondi-Heidenhain shows that they are not basi-chromatin (figures 20, 21). In the next stage the nucleus 'runs together' into a more or less compact mass in which however the chromatin and parachromatin portions are usually distinguishable. Very often the latter in some globular form adheres to the chromatin mass as though 'squeezed out' (achromatic body of Hermann).

There are many forms of the degeneration picture. Quite common is the typical chromatolytic—or preferably and more correctly karyolytic—nucleus described by Heidenhain ('90) in which the chromatin collects peripherally as a shell, series of globules, or very often a crescent, the achromatic mass being central. Figure 9 shows one of these inadequately while figures 18 and 19 give them in more detail. The condensation and corresponding shrinking of the nucleus during this stage is usually excessive so that a considerable space intervenes between the nucleus and the cytoplasm, which throughout appears scanty and consists largely of a peripheral layer which may be connected with the nuclear mass by strands.

Further changes consist in the dissolution by fragmentation (and liquefaction?) together with a loss of staining power with basic stains. The resulting mass of granular and globular debris contains much fat which is undoubtedly responsible for many of the vacuoles apparent in figures 7 and 8.

Significance. In considering the significance of these degenerations the first thought would be that they were pathological—a result of an infection or an abnormality introduced into the life conditions (lack of oxygen, insufficient circulation, etc). This, indeed, is the interpretation of Drüner, who believed that the degenerations were caused by protozoa infesting the nucleus and causing their degeneration. His figures and descriptions however are not conclusive and in the absence of any experimental evidence and in the light of the occurrence in *Desmognathus* of these degenerations, at a specific time and in a specific region such an interpretation becomes highly improbable. Likewise there is no indication of interference with the blood supply which might cause their degeneration secondarily.

On the other hand, there is much that indicates that they are *physiological degenerations*. The constancy with which they occur, the definite time of year at which they are most abundant, and especially the location in the testis and the position in the spermatogenetic cycle. This last suggests to us that they bear a relation to the regulation of the spermatogenetic process. Such a regulation appears not to have received much consideration. The investigator's attention is usually so riveted upon the intensely interesting intracellular processes involved in the periods of spermatogonial multiplication, synapsis, reduction, etc., that the possibility that these processes may be in some way coördinated with the life-habits of the form is generally not discussed. Nevertheless, it will be seen that spermatogenesis is correlated with the mating habits in those forms at least which mate at a definite season. If the multiplication of the spermatogonia and transformation into spermatocytes I is initiated, accelerated, retarded or checked at a definite stage, it can mean nothing else than that these processes are regulated growth processes similar to those that lead to the establishment of definite body form. Whether this regulation is intrinsic—within the germ cells themselves—or extrinsic is a question for the consideration of which there is as yet little basis of fact although analogy would suggest the latter. Possibly the emptying and degeneration of the first maturing lobules and the growth of the interstitial cells accompanying this may be a factor. Degenerations, therefore, so closely associated with a break in the continuity of the growth process of spermatogenesis seem associated with its regulation, and this view is strengthened by the fact that only the germ cells, not the follicle nuclei, undergo the histolytic change.

Relation to synizesis. The marked contraction of the nuclear contents into a more or less homogeneous mass which has been described in so many plant and animal forms as occurring at about the beginning of the growth period of the spermatocyte I so closely resembles in its extreme form the rounding off of the nuclei entering on the degeneration changes above described as almost to force a comparison, and one of us (Kingsbury, '02), struck by the strong general resemblance, made a suggestion that

introduces for consideration in this connection one of the critical stages of oögenesis and spermatogenesis which appeals to the writers as one of the most difficult of interpretation—the so-called *synapsis stage*.²

The suggestion then tentatively made³ was that the resemblance between the nuclei in synizesis and the degeneration under consideration might be more than a superficial one, especially as they both occurred at about the same time, associated apparently with the end of the multiplication period; that synizesis itself might possibly represent a 'running out of the spermatogonial stock.' Synizesis would on this interpretation be a 'beginning degeneration'—with recovery, which passes over later in the season into a degeneration leading to dissolution.

The following diagram or schema may serve to illustrate for the form *Desmognathus* the comparison of synizesis and the degenerations in question. Successive stages in the spermatogenesis being indicated by the letters of the alphabet as given in the legend below, while the idealized zones of the testis are numbered from 1 to 10.

² The terminology employed in the discussion of this period of the gametogenesis possibly calls for brief comment. *Synapsis* is used in the original sense of the pseudo-reduction in the chromosome number interpreted as due to a joining together in pairs. It is therefore as used here equivalent with conjugation and syn-desis. For the contraction of the nuclear contents the term *Synizesis* introduced by McClung is employed.¹ While synapsis and synizesis are usually reported as occurring together at the beginning of the growth period of the spermatocyte, after the last spermatogonial division, they are not in all cases so assigned. Montgomery places synapsis in the telophase of the last spermatogonial division. Miss King described it in the toad as occurring after the growth period of the spermatocyte, etc.

³ Page 108. "(c) when the spermatogonia cease to undergo transformation into spermatocytes in the summer, the last cysts of spermatogonia apparently undergo a chromatolysis and solution, and the boundary between the spermatocytes which are to form spermatozoa that season and the spermatogonia remaining over until the next summer, is thus well marked." Page 111. "It is suggested therefore that the contraction figures [i.e., synizesis], instead of being constructive and a fundamental phenomenon in the formation of the spermatocyte, may be an expression of a 'running out' in the spermatogonium stock and represent a tendency toward degeneration. We know as yet too little of the occurrence of the contraction figures in different forms to draw any general conclusions; possibly quite different phenomena may be here included."

With the horizontal axis representing the 'spermatogenetic wave' and the vertical axis the successive transformations with advancing season, oblique lines upward and to the right would give the similar stages at different seasons. The 'boundary plane,' when it appears, breaks the continuity of such lines. Allowing for the equalization of all stages in duration and extent which such a schema necessitates, it nevertheless gives a good diagrammatic representation of the process of spermatogenesis in the testis of Desmognathus as is indicated by comparing figures 1, 2, 6 and 3, from the months of May, June, August and September. To these might be added many others from the yearly cycle. It will be seen the synizeses (*E*) in front of the 'boundary plane' is in line with the degenerations (*D*) behind the plane.

Spermatogenetic wave											
	1	2	3	4	5	6		7	8	9	10
Advance of season	n	m	l	k	j	i	*	D	c	b	a
	n	m	k	j	i	h	*	D	c	b	a
	n	m	j	i	h	g	*	D	c	b	a
	n	m-l	i	h	g	f	*	D	c	b	a
	n	m-l	h	g	f	E		c	b	a	a
	n	m-l	g	f	E	c		c	b	a	a
	n	l	f	f	c	c		b	a	a	a

a—primary spermatogonia
c—secondary spermatogonia
E—synizeses
g—secondary spermatocytes
i—transforming spermatids
k—nearly mature spermatozoa
m—spent lobules (degenerating)

b—secondary spermatogonia
D—degenerations
f—primary spermatocytes
h—spermatids
j—maturing spermatozoa
l—mature spermatozoa
n—degenerated lobules

*—boundary plane

A second possible interpretation of synizesis that occurred to the writers when considering the resemblance of the degeneration figures to extreme synizesis, has been elaborated by Hertwig ('03); that is, that synizesis and synapsis represent an abortive mitosis. According to this view, on the one hand, synizesis represents an 'attempt on the part of' the spermatogonia to divide again—which fails; while, on the other hand the reputed conjugation of chromosomes occurring at about this time is but the imperfect fission and subsequent fusion of daughter chromosomes of such abortive division. There promises to be some time before there is any complete agreement as to the facts, let alone interpretation.

As far as synizesis is concerned, the extent of the contraction of the nuclear contents seems to vary, from a condensation in which no detail of structure is discernible, to a *tendency* only, on the part of the nuclear structures to withdraw from the nuclear membrane. Since Meves ('07), in his recent rather severe critique of the synizesis and synapsis problem, is forced to admit such a tendency at this stage of the growth of the spermatocyte, synizesis must represent a real alteration of conditions, and is not an artifact due to imperfect penetration or fixation. In Desmognathus, we still locate synizesis in the beginning of the growth period of the spermatocyte. The contraction of the chromatin in many specimens is not marked so that in many of the preparations it can be interpreted as little more than a 'tendency' to contraction. Furthermore, as was stated by Kingsbury ('02), synizesis is only well marked in the early summer, among the last spermatocytes to enter upon the growth period that season. In this connection figure 4 and particularly figure 7 may be examined, as well as the more enlarged figures 8 and 9 which are, however, not particularly characteristic and are not introduced

The suggestion was too briefly stated at that time to be easily interpreted. The idea intended to be conveyed however was that in the 'play of forces,' whatever their character, which determine the succession of spermatogonial divisions, the termination of the period of multiplication must be thought of as due to a checking of, or a loss of a power of nuclear synthesis—a 'running out,' as if from an exhaustion of 'material' which necessitates a long growth period—or leads to degeneration. The suggestion was hardly intended to have the force of a 'claim' as Miss King ('08) states it. B. F. Kingsbury.

in illustration of the synzesis figure in Desmognathus. The limitation of synzesis to this period of the year is not due to the fact that at this time the "testis contains relatively more cells in this particular stage of development" as has been intimated might be the case (King, '07). To appreciate this it is necessary to keep in mind the 'polarity' of the urodele testis which permits a very exact location of given stages and determination of their sequence. Thus the examination of longitudinal sections of organs secured throughout the spring shows in each a succession of cysts filled with cells in stages grading from the spermatogonia to mature and dividing spermatocytes. As has been said, it is only after the 'boundary' limiting that season's production is well marked that definite instances of synzesis appear—unless indeed, the isolated cysts of cells with markedly contracted nuclei that are found in testes from the spring months before the boundary plane appears represent synzesis. In this event, the cells recover and are not degeneration figures.

As far as synapsis (syndesis) or the 'conjugation of the chromosomes' is concerned, it appears to be lacking in Desmognathus. We have carefully reexamined the question in extensive material and fail to find any indication of a fusion of the chromosomes, parallel or end to end, or, indeed, of a splitting of the chromatin threads in the early growth period of the spermatocyte. The splitting of the chromosomes of the spermatocyte I in preparation for the first division appears quite early; but it becomes more and more distinct and complete as the division is approached. The changes of the growth period of the spermatocyte I occur essentially as already described by Kingsbury ('02).

Whatever general agreement may be ultimately reached as to the *facts*, i.e., the general occurrence of a union in the spermatocyte (or spermatogonial anaphase) of distinct chromosomes, end to end or parallel, and the prevalence of a contracted condition of the nuclear contents—the *explanation* of the phenomena remains quite distinct, nor should it be confounded with whatever teleological significance may attach thereto. Thus such an hypothesis as the abortive mitosis interpretation of the synapsis period by R. Hertwig seems particularly suggestive, since it pre-

sents the possibility of an explanation on the basis of a general interpretation and treats the cell as a unit.

Synizesis, as an alteration in the morphology of the nucleus, can be adequately approached only by a consideration of the 'play of forces'⁴ upon which the morphology of the nucleus depends and in which a correlation with the cytoplasm must be intimately involved. An adequate analysis of such forces has not, as far as we are aware, been made. One is particularly impressed with the existence of such forces when in karyolysis, as a result of their suspension, the nuclear substance is free to follow the (simpler) laws of its physical state and condense into spherical masses. It is this which suggested that synizesis expressed a more or less complete suspension of nuclear processes. Since the contraction is toward the idiosome the impression is strong that, in the contraction, the relations of the nucleus to that portion of the cytoplasm in which the idiosome is, persist or exist, possibly in exaggerated form, while there is a more or less complete suspension of nuclear-cytoplasmic relations over other portions of the nuclear membrane.

The arrangement of the chromatin (chromosomes) oriented in relation to the idiosomatic cytoplasm in the well known 'bouquet stage' indicates that such a peculiar interrelation between the nucleus and this portion of the cytoplasm exists (persists) throughout the growth of the spermatocyte I.

Suggestions of such important correlations are naturally to be found in the literature. Thus, Winiwarter ('08) recognized synizesis as expressing a correlation between the chromosomes and the idiosome, the latter exerting a real influence of attraction upon the chromatin filaments, but affecting the cytoplasm as well, since the mitochondria cluster around the idiosome. As expressing the attraction he proposes the term *centrotaxis*, but of its nature we are entirely ignorant as yet. Montgomery ('11) suggests that synizesis, which he finds may occur during a large portion of the growth period of the spermatocyte in *Euschistus*

⁴ By this somewhat unsatisfactory expression is meant the sum total of forces that are undoubtedly operative in protoplasm—electrical attractions and repulsions, chemical affinities and reactions, osmotic tensions, etc.

indicates possibly a rhythmic discharge of material from the nucleus. The chromatin plate described by him likewise is indicative of an idiosome-nuclear correlation.

Practically nothing is known regarding the frequency of occurrence in amphibia other than *Salamandra* and *Desmognathus*, of degenerations similar to those described. Through the kindness of Dr. Montgomery sections of the testis of *Plethodon cinereus erythronotus* were examined and comparable degenerations were found to be present. Likewise they have been seen in the testis of *Salamandra atra*. In these forms, however, no systematic study of the degenerations has been made in which there has been seriously attempted the ascertainment of any definite relation to the process of spermatogenesis, the stage at which they occur, their relation to the annual cycle or their location within the testis, nor has the relation of the spermatogenetic process to the testis been studied.

Miss King ('07) has found no trace of such degenerations in *Bufo*. She says: "I have never found a condensation of the chromatin in the spermatogonia as Kingsbury has described for *Desmognathus*, and I am unable to confirm his statement that 'contraction figures do not occur constantly in spermatocytes.'" To this the following comments may be made; first, that work upon one form cannot be relied on for confirmation or disproof of work done upon another form. The spermatogenetic process seems to be worked out in the anuran testis in a way quite different from that prevailing among the urodeles. In the toad it is apparently intralobular; many different stages are found within the confines of a single lobule.⁵ The seriation of stages in such a testis as the toad's are much more difficult, and, it may be ventured, karyolytic nuclei might easily be overlooked as they would probably occur singly. This, however, from Miss King's careful

⁵ Cf. King, '07, p. 346. "As a rule all of the cells in a cyst are in approximately the same stage of development, but a single follicle [lobule] may contain both spermatogonia and maturing spermatids. A transverse section of the testis, therefore, shows practically all stages in the development of the spermatozoa." In *Desmognathus* in a transection all cells would be in approximately the same stage, while in a single longitudinal section at the right season of the year, practically every stage might be seen.

study would hardly seem likely and it is far more probable that if such degenerations occur, they do so at a later season than that studied by Miss King (i.e., after September), possibly at the beginning of hibernation, when, if ever, one might expect a checking of the spermatogonial divisions to be accompanied by degenerations. Granted that these are 'physiological degenerations,' it should be appreciated that there is no reason for believing that the factors upon which they depend would be operative in all forms in the same way. The degenerations might or might not occur, which fact should be considered in making comparisons between the processes taking place in the testis of the toad and in that of the salamander where this may be particularly applicable.

The lavish outlay of germ cells and their wholesale degeneration has been commented on by a number of writers. There may be particularly mentioned: Winiwarter and Saimont (mammals), Hoffman ('92), D. Hollander ('05) (birds), Bouin ('01), Dustin ('07), Levi ('05) (amphibia).

The descriptions of Winiwarter who has given the most monographic description of mammalian oogenesis are particularly interesting. In the rabbit ('00) he described two epochs of degenerations which were completely separated. Of these the first, including typical and atypical karyolysis, is of particular interest in this connection. The second occurred in the atresia folliculi. Winiwarter found that the multiplication of the oogonia ceased soon (about ten days) after birth. The degenerations of the first epoch extended from the twenty-third day embryo nearly to eighteen days after birth. The degenerating cells were found to be, in the large majority of cases at least, oogonia which succumbed particularly at the time of their division. At what point the karyolysis sets in he is not sure but he thinks that in all probability it is at the equatorial plate stage. Comparison of his results with the conditions in *Desmognathus* are quite suggestive.

In the cat ('08) the degenerations begin to appear singly. In embryos of forty-five to fifty days large groups of degenerating cells are present. Shortly after birth the multiplication of the oogonia suffers an arrest and, coincident with this, there is a recrudescence of degeneration. The groups of nuclei particularly

affected are his poussieroides and transitory (i.e., presynaptic). The change shows itself first in the nuclei; the fine network or fine granulations forming larger masses grouped around the nucleolus.

CONCLUSION

In *Desmognathus fusca* at the time that the transformation of spermatogonia into spermatocytes ceases, degeneration figures in large numbers, involving whole cysts and lobules, may be found among the cells that have 'failed' to transform that season. They have a definite position in the testis as well as in the spermatogenetic cycle and seem to be closely associated with the regulation of the spermatogenetic process.

Apparently similar degenerations have been reported in a number of different forms, in the oogenesis and in the spermatogenesis.

Such degenerations have undoubtedly a greater significance in connection with the activities of the reproductive organs than is generally recognized.

For their adequate treatment, however, spermatogenesis must be dealt with in its relation to the whole organ and the whole organism. Little help in interpretation, it is believed, may be expected from the ultra-chromosomal point of view, or even from that of the cell theory.

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LITERATURE CITED

- BIZZOZERO 1888 Anwendung des Methylgrünes zur Erkennung der chemischen Reaktion und des Todes der Zellen. *Virchow's Archiv f. pathol. Anat.*, Bd. 113.
- BOÛIN, M. P. 1901 Histogenese de la glande genitale femelle chez *Rana temporaria*. *Arch. f. Biol.*, T. 17.
- 1903 Spermatocytes en degeneration utilizes comme material alimentaire pendant la spermatogenese. *Compt. rend. Soc. Biol.*, T. 55.
- DRÜNER, L. 1894 Beiträge zur Kenntnis der Kern- und Zellen-degeneration und ihre Ursache. *Jenaische Zeitschr. f. Naturw.*, Bd. 28.
- DUSTIN, A. P. 1907 Recherches sur l'origine des gonocytes chez les Amphibiens. *Arch. de Biol.*, vol. 23.
- FLEMMING, W. 1885 Ueber die Bildung von Richtungsfiguren in Säugetiereiern beim Untergang Graafischer Follikel. *Arch. f. Anat. u. Entw.*
- 1887 Neue Beiträge zur Kenntniss der Zelle. *Arch. f. mikr. Anat. Th. I.*, Bd. 29.
- HEIDENHAIN, M. 1890 Beiträge zur Kenntniss der Topographie und Histologie der Kloake. *Arch. f. mikr. Anat.*, Bd. 35.
- 1892 Ueber Kern und Protoplasma. *Festschr. f. Kölliker*.
- HERMANN, L. 1888 Ueber regressive Metamorphosen des Zellkerns. *Anat. Anz.*, Bd. 3.
- 1890 Beiträge zur Histologie des Hodens. *Arch. f. mikr. Anat.*, Bd. 34.
- HERTWIG, R. 1903 Ueber Correlation von Zell- und Kerngrösse und ihre Bedeutung für die geschlechtliche Differenzierung und die Teilung der Zelle. *Biol. Centralbl.*, Bd. 23.
- HOFFMANN, C. K. 1892 Étude sur le developpement de l'appareil urogenital des oiseaux. *Verh. d. Koninkl. Akad. van Wetensch. Amsterdam*, sec. 2, vol. 1.
- D'HOLLANDER, F. 1905 Recherches sur l'oogenese et sur la structure et la signification du noyau vitellin de Balbiani chez les oiseaux. *Arch. d. Anat. micr.*, T. 7.
- KING, HELEN DEAN 1907 The spermatogenesis of *Bufo lentiginosus*. *Am. Jour. Anat.*, vol. 7.
- 1908 The oögenesis of *Bufo lentiginosus*. *Jour. Morph.*, vol. 19.
- KINGSBURY, B. F. 1902 The spermatogenesis of *Desmognathus fusca*. *Am. Jour. Anat.*, vol. 1.
- LEVI, G. 1905 Sulla differenziazione del gonocita e dell' ovocita degli Anfi con speciale riguardo alle modificazione della vescicola germinativa. *Arch. Ital. di Anat. e di Embriol.*, T. 4.

- McCLUNG, C. E. 1905 The chromosome complex of Orthopteran spermatocytes. Biol. Bull., vol. 9.
- McGREGOR, J. H. 1899 The spermatogenesis of *Amphiuma*. Jour. Morph., vol. 15.
- MEVES, FR. 1895 Ueber eigentümliche mitotische Prozesse in jungen Ovocyten von *Salamandra maculosa*. Anat. Anz., Bd. 10.
- 1907 Die Spermatocytenteilungen bei der Hönigbiene (*Apis mellifica*, L.), nebst Bemerkungen über Chromatin-reduktion. Arch. f. mikr. Anat., vol. 70.
- 1911 Ueber die Beteiligung der Plastochondrien an der Befruchtung des Eies von *Ascaris megalocephala*. Arch. f. mikr. Anat., Bd. 76.
- MONTGOMERY, T. H. 1903 The heterotypic maturation mitosis in Amphibia and its general significance. Biol. Bull., vol. 4.
- 1906 Some observations and considerations upon the maturation phenomena of the germ cells. Biol. Bull., vol. 6.
- 1911 The spermatogenesis of an hemipteron, *Euschistus*. Jour. Morph., vol. 22.
- SCHMAUS, H., und ALBRECHT, E. 1894 Degenerationen von Mitosen. Ergeb. d. allg. Pathol., Bd. 1.
- 1894 Physiologische Degenerationen. Ergeb. d. allg. Pathol., Bd. 1, 2te. Teil.
- WINIWARTER, H. v. 1900 Recherches sur l'ovogenese et l'organogenese de l'ovaire des Mammiferes (Lapin et Homme). Arch. de Biol., T. 17.
- WINIWARTER, H. v. et SAIMONT, G. 1908 Nouvelles recherches sur l'ovogenese et l'organogenese de l'ovaire des mammiferes (Chat). Arch. de Biol., T. 24, Ch. 4.

PLATE 1

EXPLANATION OF FIGURES

1 Longitudinal section of testis of *Desmognathus fusca*. May 24; fixed in Hermann's fluid; iron hematoxylin stain. From the primary spermatogonia in the portion at the bottom of the photograph, one passes up through a gradual succession of stages to spermatids at the top of the figure. The nearly mature spermatocytes just below them may be distinguished by their larger size. The boundary between the cells destined to furnish spermatozoa that season and the residual cells, is not yet evident in this specimen.

2 Longitudinal section, testis *Desmognathus fusca*, fixed June 7, in Hermann's fluid; iron hematoxylin stain. The interstitial cells about the degenerated lobules occupy the upper portion, below them come developing spermatozoa; at the lower end, spermatogonia. The boundary is becoming well marked. Enlarged photograph of the boundary region is shown in fig. 4.

3 Longitudinal section, testis *Desmognathus*, fixed September 7 in Hermann's fluid; iron hematoxylin. The body of the testis occupied by spermatozoa, the lower portion by spermatogonia. The boundary is thus conspicuous. Fig. 5 shows an enlarged view of the boundary. An intermediate stage is shown in fig. 6.

4 Testis *Desmognathus*. Photograph giving an enlarged view of the boundary region of fig. 2. Spermatocytes occupy the upper lobules, spermatogonia the lower lobules, the separation being particularly clear on the right side. Three lobules of spermatogonia at the boundary region show more or less extensive karyolysis.

5 Testis of *Desmognathus*. Photograph giving an enlarged view of the boundary region of fig. 3. Spermatozoa occupy the lobules above the boundary, spermatogonia those below. Degenerations are seen in one entire lobule and in portions of two others.

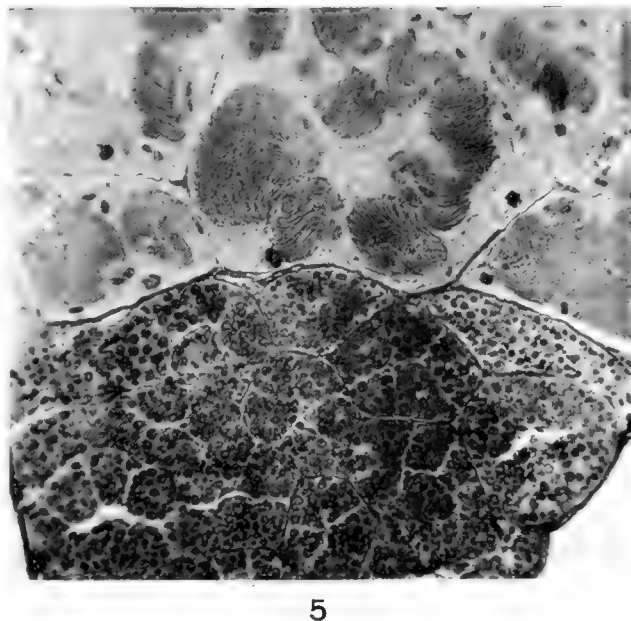
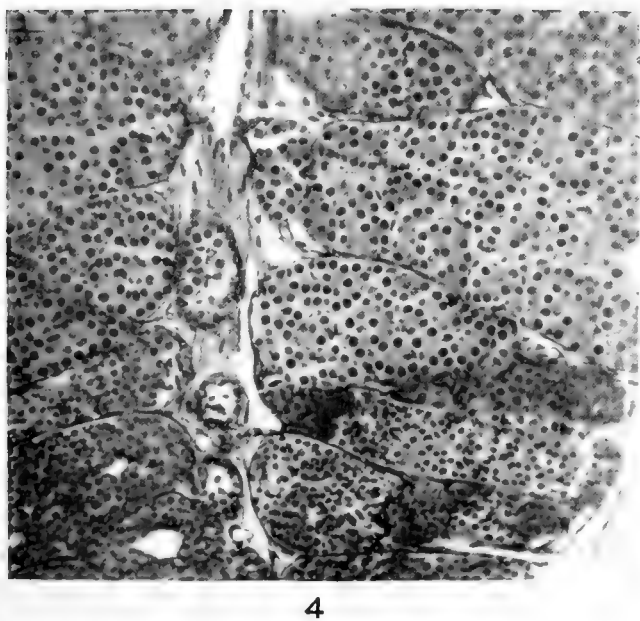
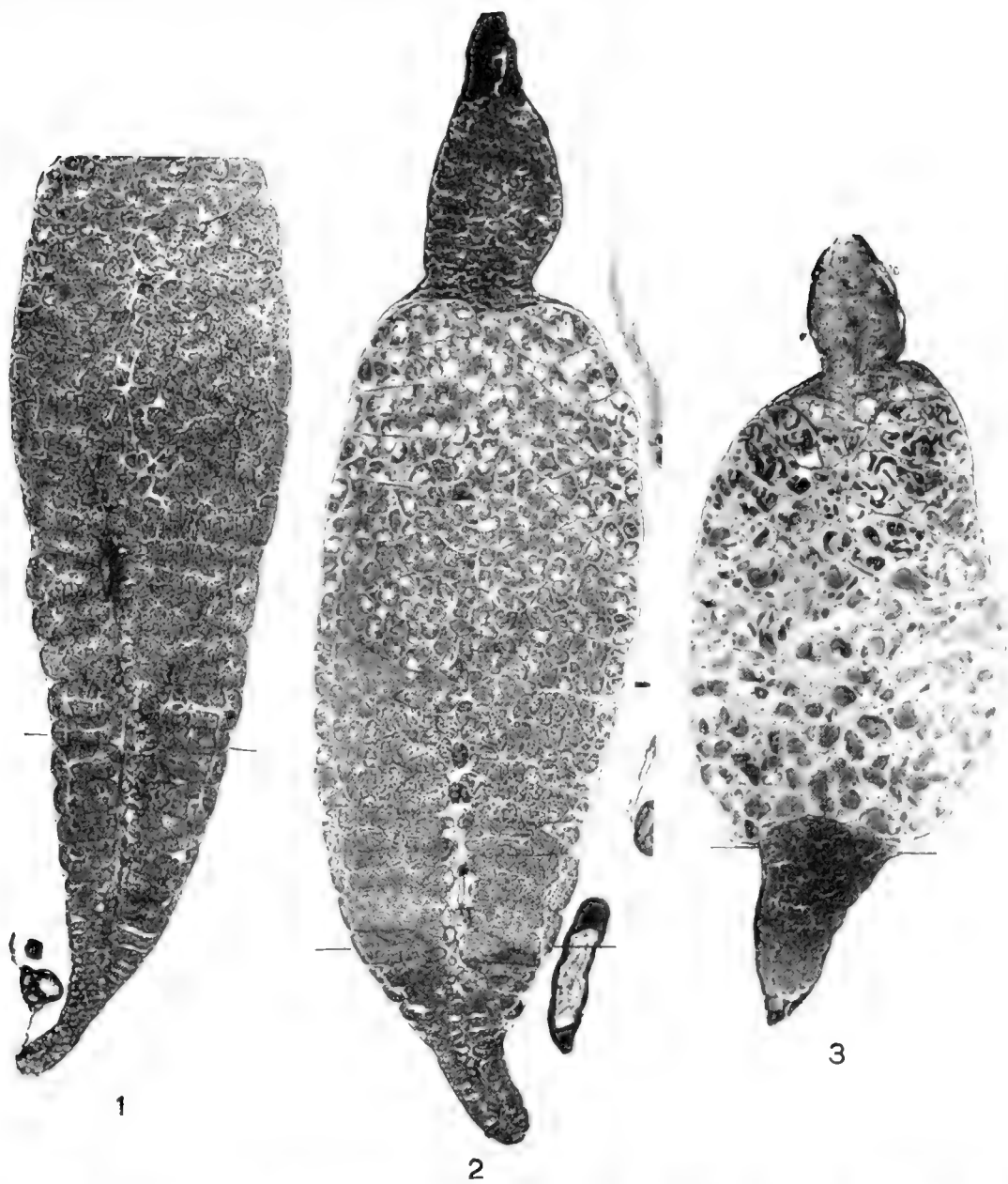


PLATE 2

EXPLANATION OF FIGURES

6 Testis of *Desmognathus*. Longitudinal section. Fixed in Hermann's fluid August 21; iron hematoxylin stain. Two lobes are shown, the lower end of the one on the right being connected with the lower end of the one on the left. In the larger lobe, the region of degenerated lobules occupies in the figure the upper end. These are succeeded below by spermatozoa, maturing spermatozoa, transforming spermatids, spermatids, spermatogonia, the boundary between the regions occupied by the last two being well shown in both lobes.

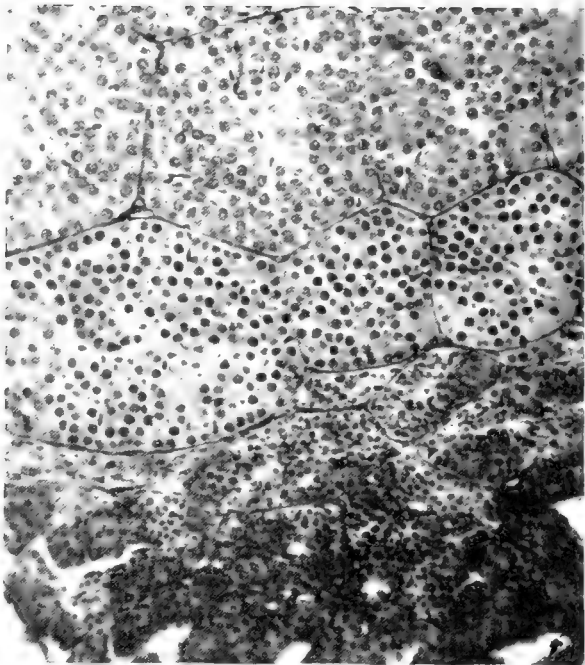
7 Testis of *Desmognathus*. Longitudinal section. Fixed June 7 in Hermann's fluid; iron hematoxylin stain. View of the lobules in the boundary region. In the upper three lobules are growing spermatocytes I; the intermediate three lobules show synizesis; below these comes the boundary and lobules of spermatogonia among which many cysts are undergoing degeneration.

8 Testis *Desmognathus*. Photograph of the boundary region, showing a degenerating lobule, filled with debris, fat vacuoles, karyolytic nuclei and the normal nuclei of the follicle cells. Above the degenerating lobule the spermatocytes I indicate a slight condition of synizesis. The lobule below contains spermatogonia.

9 Testis of *Desmognathus*. Photograph similar to that shown in fig. 8, from the surface of the testis (on the right side of figure). One degenerating lobule is shown and portions of two others contain degenerating cysts.



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PLATE 3

EXPLANATION OF FIGURES

10 Testis of *Desmognathus*. Photograph at high magnification (2 mm. apochromatic, no. 2 Zeiss projection ocular) of a portion of a lobule of spermatogonia in which one cyst is in an early stage of degeneration. The resemblance to marked synizesis is striking.

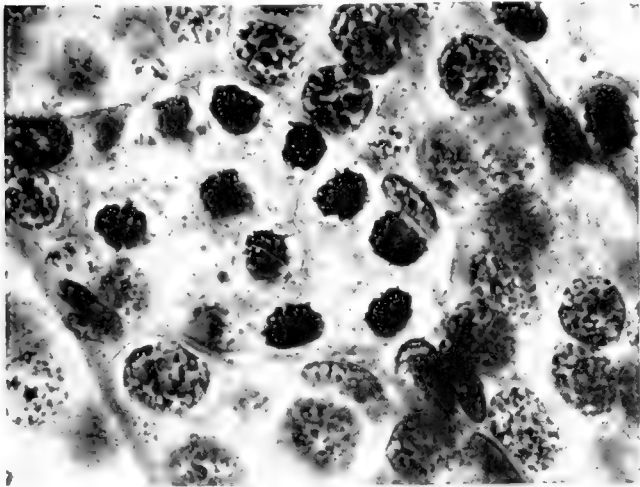
11 Testis of *Desmognathus*. Photograph at high magnification (2 mm. apochromatic objective no. 2 Zeiss projection ocular). To show the karyolytic nuclei in a degenerating lobule and the normal nuclei of the follicle cells. The lobules above contain spermatozoa; those below dividing spermatogonia.

12 Testis of *Desmognathus*. A cyst filled with karyolytic cells. The three nuclei in the neighboring cyst (upper left hand corner) are probably just about to enter upon degeneration.

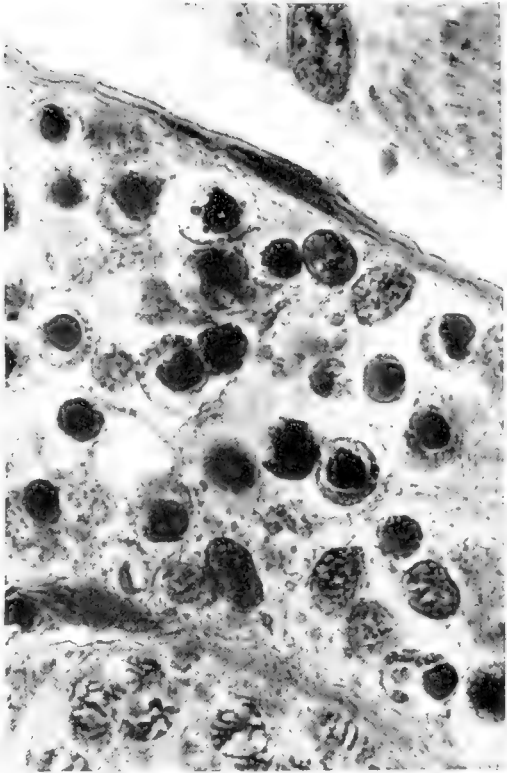
13 Testis of *Desmognathus*. Photograph from a lobule of degenerating spermatogonia.

14, 15, 16, 17 Pen and ink drawings of camera lucida sketches of typical karyolytic cells. The resemblance to the extreme synizesis nuclei described in other forms is striking.

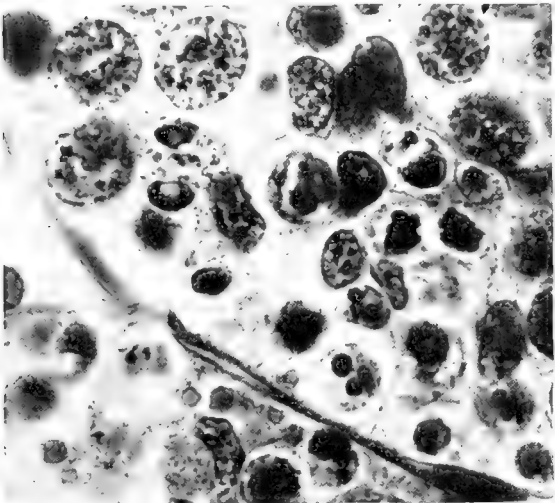
18, 19, 20, 21 Colored drawings from camera lucida sketches of typical karyolytic cells. From preparations stained with the Ehrlich-Biondi-Heidenhain triple stain.



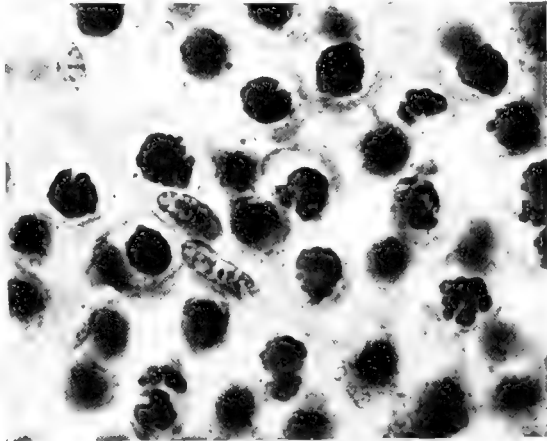
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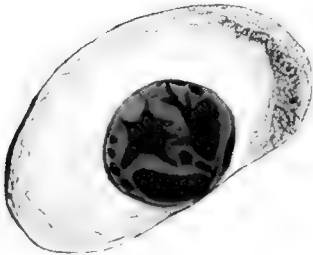
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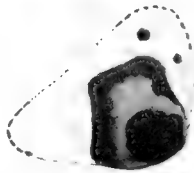
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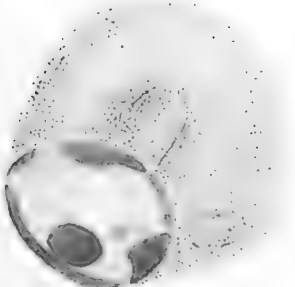
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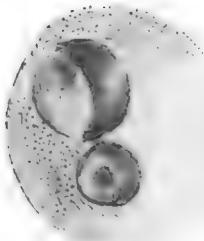
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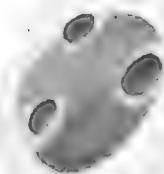
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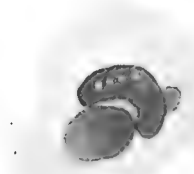
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21

THE EPITHELIUM OF TURBELLARIA

R. T. YOUNG

From the State University of North Dakota

SIX FIGURES

The existence of an epithelium in trematodes and cestodes has been a much debated question for many years, while among those who deny the presence of this tissue in these worms there is much difference of opinion as to the origin of this condition, some maintaining that the epithelium has been lost, some that it has been metamorphosed into the cuticula, while others fail to express an opinion on this point.

If we turn to the Turbellaria, the probable ancestors of trematodes and cestodes, we find conditions which I believe give a clue to the answer to this question. Most of these possess a typical nucleated epithelium, the cell boundaries of which however are difficult to observe without special methods; while in the pharynx and its sack nuclei are often lacking in the epithelial layer, having invaded the parenchyma to form an insunken epithelium as has been experimentally observed by Jander ('97) in the regenerating pharynx of *Dendrocoelum*.

In many forms the general epithelium presents conditions similar to those common in the pharynx, while in some cases it has been claimed that neither nuclein or cell boundaries are demonstrable even with special methods of technique. Thus Böhmig ('90) in several species of *Alloeocoela* was unable to demonstrate cell boundaries in the epithelial layer by treatment with silver nitrate, while the distribution of nuclei was very irregular.

The typical conditions in the turbellarian epithelium are well represented in *Planocera inquilina* (fig. 1). The surface of this

worm is covered by a layer of cilia averaging 5.7μ in thickness dorsally and 5.4μ ventrally.¹ In fixation, they become matted together to form a tangled mass, in which it is difficult to observe individual cilia. Where they are inserted in the epithelium, the characteristic basal swellings give the appearance of a thin dense layer at the surface, which has been interpreted by various writers as a cuticula. The epithelial cells have a fibrillar structure presenting numerous small spaces² which are very likely the result of shrinkage.

The course of the fibrillae is more or less irregular, though in a general way perpendicular to the surface, producing the striations mentioned by various authors in the turbellarian epithelium. They form a close network, varying in density from point to point, in the meshes of which lie the spherical or ovoid nuclei with a distinct chromatic network and a definite membrane which stains similarly to the latter, and in most, but not all cases, appears to be complete. Frequently, but not always, the network is condensed at one or more points to form false nucleoli. This appearance may be the result of shrinkage. These nuclei average 4.4 by 5.5μ in diameter.³ Besides nuclei, numerous rhabdoids occur in the epithelium, an account of which does not concern us here. The fibrillae appear to be continuous with basal extensions of the cilia, but on this point I cannot speak positively.

Cellular outlines, faintly evident in surface views, are indistinguishable in cross sections.⁴ I have not observed a differentiation of epithelial and interstitial cells, as described by Lang ('84) for polyclads. Nor can I distinguish the difference in size of nuclei which he describes and figures.

¹ Average of seven measurements. Variations in thickness of the ciliary layer of from 3 to 8μ occur. These are probably not altogether normal, but where indications to the contrary are lacking I have included them in my averages.

² Except in the denser surface layers.

³ Average of twenty measurements.

⁴ Occasionally I find an apparent fibrilla passing through the epithelium from outer to inner surface of the latter. These may represent cell boundaries but they are distinguishable from other fibrillae only by their extent from surface to surface of the epithelium and by their more nearly vertical direction. In some places where shrinkage has occurred apparent cell outlines may be seen.

The thickness of the epithelium as well as the shape of the nuclei is largely dependent on the state of expansion or contraction of the worm. Dorsally it averages 10μ , ventrally 8.7μ in thickness.⁵

Directly beneath the epithelium is the basement membrane. This is in most places a well marked layer averaging 2.7μ in thickness dorsally and 1.5μ ventrally,⁶ but varying from 4μ to a mere line from point to point. Toward the edge of the body it becomes very thin. Next to the epithelium the membrane frequently shows a very distinct outline, on the inner side it is less sharply differentiated from the parenchyma. It is evidently differentiated chemically from both parenchyma and epithelium, judging by the difference in stain between it and these latter tissues. In haematoxylin-eosin preparations, the latter are stained light blue or gray, while the basement membrane is straw-colored, being thereby very distinctly marked off from the other tissues. In sections taken perpendicular to the surface, the basement membrane appears nearly homogenous, but where the sections are oblique or parallel to the surface a fibrillar structure is plainly visible. The course of the fibrillae, while more or less irregular, is in general parallel to the surface and thus at right angles to those of the epithelium. A continuity between them and those of the parenchyma on the one hand, and the epithelium on the other, I consider probable although I am unable to demonstrate it positively.

Jander ('97, p. 24) describes the origin of the basement membrane as a "Verdickung der Netzstränge bis zu dem Maasse . . . die eine Basalmembran darstellt." The same author (l.c., p. 27) describes the striations of the epithelium as occasionally passing "durch die Basalmembran bis in die äussere Längsmusculatur hinein," but qualifies this statement by adding that "ist es auch nicht unmöglich irrtümlicher Weise einen derartigen Zellplattenstreif in einen Bindegewebsstrang zu verlängern." This view is supported by my own observations of the fibrillar nature of this membrane and its probable continuity with

⁵ Average of seven measurements varying from 4 to 14μ .

⁶ Average of seven measurements.

the parenchyma, and by its replacement in *Polychaerus caudatus* by a fibrous network directly continuous, through the sub-epithelial muscle layers, with the parenchyma, as is the case in *Taenia serrata* and its larva (Young, '08). Woodworth ('91, p.20) on the contrary believes the basement membrane of *Phagocata gracilis* to be a hypodermal product. In the fibrillar groundwork, some deposit is probably formed, either by the epithelium or the parenchyma, or by both, which intimately unites the fibrillae into a homogenous mass, and is the cause of the differential staining capacity of this membrane as described above. Nuclei in the basement membrane, as described by Lang ('84) are not present here.⁷

Conditions in general similar to the above exist in several other turbellarians studied by me (viz: *Planaria maculata*, *Dendrocoelum lacteum*, *Phagocata gracilis*, *Bothromesostoma personatum*, *Mesostoma tetragonum*, *Mesostoma* sp. and *Phaenocora*(?). In not all, however, are the nuclei as numerous as in *Planocera inquilina*. While the abundance of nuclei depends in a large measure on the condition of expansion or contraction of the worm, still by comparison of several specimens of each species similarly fixed, it is possible to construct a series with reference to nuclear abundance leading from the last named form to those in which nuclei are seldom or never found in the epithelium.

Such a form is *Polychaerus caudatus* (fig. 2) which presents an advanced stage in the development of an insunken epithelium. Beneath the cilia, which in preserved material appear to form an almost continuous layer, is a loosely fibrillar, vacuolated layer representing the epithelium, while a basement membrane is not differentiated. The fibrillae form an irregular network, showing no definite arrangement with reference to the surface, and apparently in direct continuity with that of the under-lying parenchyma on the one hand and with the bases of the cilia on the other. I cannot speak positively regarding this however. The vacuoles in the epithelium of this worm I believe are, largely

⁷ Regarding this membrane, Lang says however (l. c. p. 64) “. . . sie auf vielen Präparaten ganz homogen aussieht, weil viele für die übrigen Gewebe des Körpers treffliche Tinctionsmittel dieselbe diffus färben.”

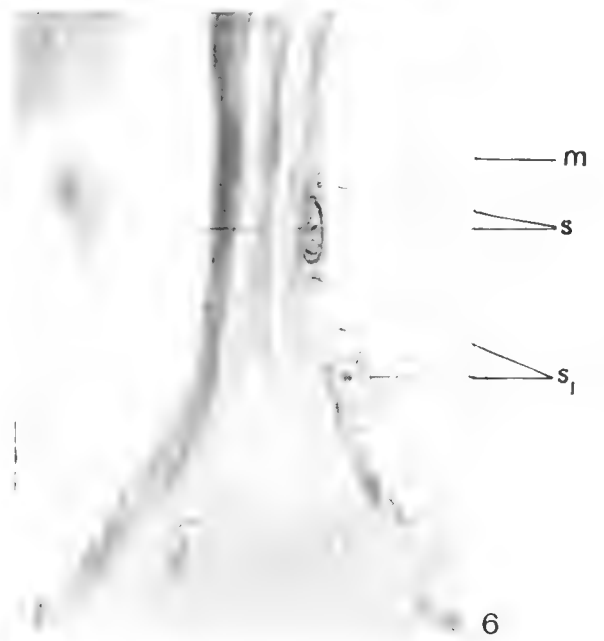
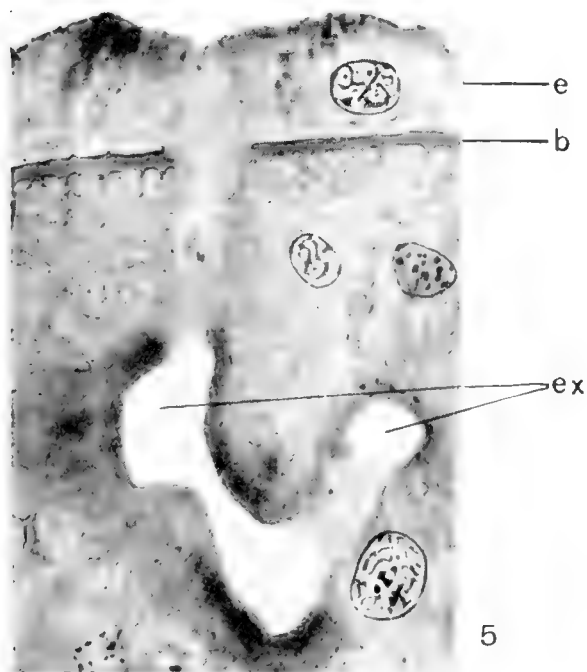
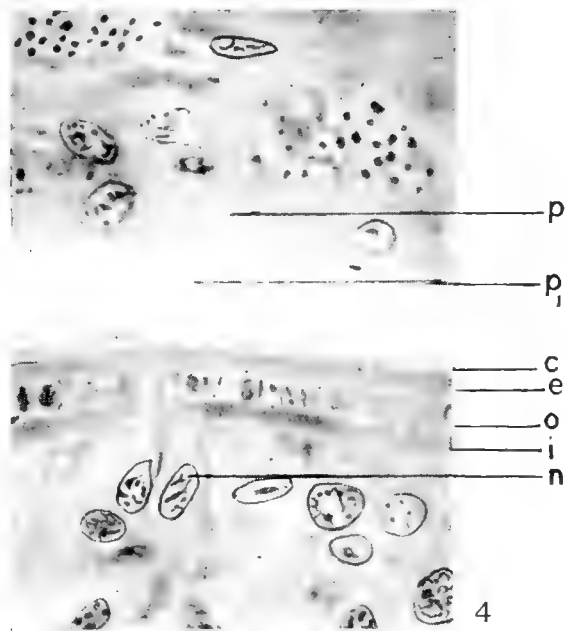
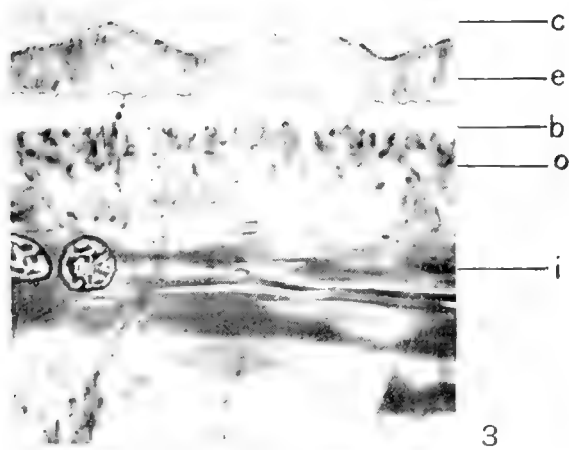
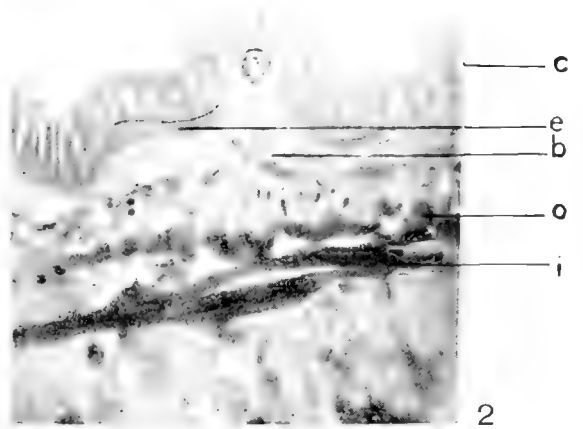
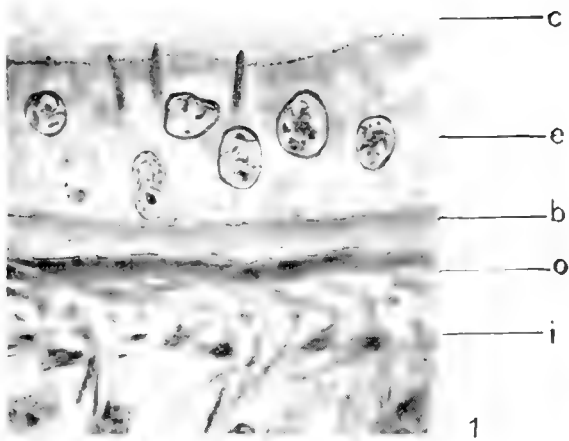
ABBREVIATIONS

<i>b</i> , basement membrane	<i>o</i> , outer muscle layer
<i>c</i> , cilia	<i>p</i> , wall of pharyngeal sack
<i>e</i> , epithelium	<i>p</i> ₁ , cavity of pharyngeal sack
<i>ex</i> , excretory duct	<i>s</i> , cavity of seminal vesicle
<i>i</i> , inner muscle layer	<i>s</i> ₁ , remnant of epithelium of seminal
<i>m</i> , muscles of seminal vesicle	vesicle
<i>n</i> , nucleus of insunken epithelium	

All photomicrographs were made on Cramer isochromatic plates through a ray filter of 2 per cent potassium bichromate, an arc light being the illuminant employed. The lens combination was a Bausch and Lomb $\frac{1}{16}$ obj. and a Zeiss No. 12 compens. oc., the camera being so adjusted as to give a magnification of 1000 diameters in each case. The positives were retouched at the microscope.

EXPLANATION OF FIGURES

- 1 Body wall of *Planocera inquilina*.
- 2 Body wall of *Polychaerus caudatus*.
- 3 Body wall of *Bdelloura propinqua*.
- 4 Wall of pharynx and pharyngeal sack of *Planaria maculata*.
- 5 Excretory duct of *Dendrocoelum lacteum*.
- 6 Seminal vesicle of *Bothromesostoma personatum*.



at least, due to shrinkage, as suggested above for *Planocera*. Occasional rhabdoids are scattered through the epithelium and ciliary layer. The main point of interest, however, in the epithelium of *Polychaerus* is the occasional occurrence of nuclei. These are found mainly dorsally, but I have observed at least one or two clear cases of their presence ventrally, one of which is shown in the figure. There can, I think, be no question as to the nuclear character of this and similar bodies in the epithelium. Their structure is identical with that of the parenchyma nuclei. They are round or oval in outline, average 4.7 by 3.5μ in diameter⁸ and contain a dense chromatic network surrounded by a fairly definite membrane, which is however obviously incomplete in some cases. It is not impossible, however, that they may owe their presence here to distortion of the tissue produced by contraction of the worm during fixation. This would explain their greater number in the dorsal epithelium where the tissues (in my preparations) are much more distorted than they are ventrally. The fact of their occurrence in the ventral epithelium, however, where but little distortion has occurred and the further fact of their occurrence in the epithelium of other Acoela as recorded by von Graff ('91) renders it probable that their occasional presence here is normal.

In *Bdelloura propinqua* (fig. 3) there appears to be an entire absence of nuclei in the epithelium examined. The surface of this worm is covered by a layer of cilia similar in appearance to that of *Planocera* already described. Below the cilia is the epithelium which also, except for the absence of nuclei, has a structure similar to that of the last named species. Here is found the same layer of basal swellings of the cilia at the points of their insertion in the epithelium, the same fibrillar network with its meshes approximately perpendicular to the surface, and containing numerous vacuoles which are probably artefacts produced by shrinkage, as they are sometimes absent or but poorly developed. Here too exists the same difficulty in determining the precise relation between the epithelial fibrillae and the cilia

⁸ Average of ten measurements.

on the one hand and the basement membrane on the other. The cilia are probably continuous with the epithelial fibrillae and possibly such a continuity also exists between the latter and those of the basement membrane.

In vertical sections, it is impossible to distinguish cell boundaries in the epithelium, but in tangential ones occasional polygonal areas may be seen which probably indicate its cellular character. Treatment with silver nitrate, moreover, reveals the cell boundaries very clearly.

Directly below the epithelium is a definite basement membrane which, in most of my material, appears homogenous, but in one specimen shows a very evident fibrillar character. The basement membrane here is very lightly stained and appears as though the ground substance had been in some way removed from the meshes of the fibrillae, thereby rendering them apparent. These form a loose network, the meshes of which run in general parallel to the surface and stain similarly to those of the epithelium. Externally, the membrane presents a scalloped appearance, which may possibly be due to the insertion of the epithelium, which in fixation has shrunk a little away from the membrane, thus bringing its scalloped appearance more plainly into view. Whether there is any anastomosis between the fibrillae of the basement membrane and those of either the epithelium or the parenchyma is a point which I must leave undecided. That this membrane is chemically different from the other tissues is a point which is indicated by its differential stain. In sections strongly counter-stained with eosin, the epithelium is red or pinkish while the basement membrane varies from colorless or light straw color to gray or pale brown.

The ciliary layer, epithelium and basement membrane show wide variations in thickness, not only in different specimens, but in different parts of the same specimen. These differences are doubtless due in great measure to differences in amount of contraction of the tissues in fixation and to distortion produced thereby; they may also be due in part to differences in the plane of section; probably also to differences in development of the

different layers. The following measurements⁹ (in μ) indicate the variations referred to:

	DORSAL	VENTRAL
Cilia.....	5, 3, 5, 3, 3	3, 7, 4, 3, 3
Epithelium.....	5, 7, 7, 3, 3	3, 4, 7, 3, 3
Basement membrane.....	5, 4, 11, 3, 3	3, 4, 15, 3, 3

At the edge of the body, where numerous glands open, the basement membrane is reduced to a very narrow band.

While nuclei are typically present in the surface epithelium of the Turbellaria, they are frequently either very rare in or entirely absent from the pharynx and pharyngeal sack, and cell boundaries can only be demonstrated by special methods, the epithelium thus assuming the appearance of a cuticula (fig. 4). The distribution of the cilia in this region is also very variable according to accounts of various authors, these being in some cases present, in others absent throughout the pharynx; in some present on its outer but not on its inner wall, while in others the reverse holds true. In general, the epithelium of the gut forms a definite one-celled layer, but in some cases (Böhmig, l.c. in *Plagiostoma bimaculatum*, *maculatum* and *sulphureum*; Fuhrmann, '98 in *Plagiostoma violaceum*), etc., it appears to be intimately connected with the surrounding parenchyma, while in the Acoela, as is well known, the gut is replaced by parenchyma, the pharynx opening directly into the latter. The oesophagus, the transition from pharynx to gut, may have an epithelium of the ordinary type, or an insunken one (Luther, '04, etc.).

The statements regarding the epithelium of the excretory ducts are again conflicting. In general an epithelium appears to be present, at least in the main ducts; but in some cases (Luther, l.c.), the wall is not sharply differentiated from the surrounding parenchyma, while a non-nucleated terminal duct in *Monoophorum striatum* is described by Böhmig (l.c.), so that the existence of a typical epithelium in the excretory system of all Turbellaria

⁹ These measurements are arranged in order from five worms, the first of each set referring to one specimen, the second to another, etc.

cannot be considered as definitely established. This uncertainty is probably due in part at least to the difficulty of demonstrating the excretory system in preserved material. Its delicacy renders its tracing, at least to the finer branches, very difficult in preserved material. So far as I have been able to find the excretory ducts in my own sections, I have seen no evidence of a definite epithelium. Their walls apparently consist of a collection of protoplasmic strands in direct continuity with those of the surrounding parenchyma, in which are imbedded occasional nuclei (fig. 5).

Conditions in the reproductive ducts and glands show considerable variation according to the statements of various authors. In general, an epithelium is present, which is, however, very variable in form. It may be insunken (penis of *Byrsophlebs nana* von Graff, '03 and *Geoplana pulla*, von Graff, '91), and may lack nuclei (penis of *Yungia aurantiaca*, Lang, l.c.), or both nuclei and cell boundaries (Typhloplaninae, Luther, l.c.). The latter author says (p. 98):

. das Epithel des Atriums geht an der Spitze des Penis in eine Kernhaltige Plasmamasse über, in der sich keine Zellgrenzen nachweisen lassen oft ist sie (in the penis) nur noch an den hier und da der Innenfläche anliegenden platten Kernen zu erkennen, in anderen Fällen gelingt es überhaupt nicht mehr ihr Vorhandensein festzustellen.

In *Plagiostomum reticulatum*, von Graff ('08, p. 2287) describes the epithelium of the "äusseren Penisrohrwandung" as "vollends cuticulaähnlich" which "färbt sich nicht mehr und erscheint vollkommen homogen: im Ductus ejaculatorius präsentiert es sich als eine haarscharfe, stark roth tingirte Linie."

According to Luther (l.c.) in the Macrostomidae, an epithelium is present in the antrum femininum alone, the gland ducts being parenchyma spaces. The same author finds in the Mesostomidae and most of the Typhloplaninae an epithelium in the virgin bursa copulatrix, which later degenerates, leaving the basement membrane superficial; the latter then becoming strongly developed. In the bursa stalk, however, the epithelium persists. The inner wall of the bursa seminalis of *Gyratrix hermaphroditus* is lined by

a plasma layer continuous with "die Vacuolisirte Ausfüllungsmasse . . . in welcher zerstreute Kerne neben Spermatamassen eingebettet sind" (von Graff, '05, p. 140). A similar condition is presented by the bursa copulatrix of *Monoophorum durum* (Fuhrmann, l.c.). Until more information is available regarding the origin of ovary, testis and yolk glands in Turbellaria, speculation concerning the presence or absence of an epithelium in these organs had better be postponed. At present, statements of different authors are divergent, and in many cases not specific, on this point, some, as Lang (l.c.), claiming its existence, while this is denied or not mentioned by others.

In some places (pharyngeal sack, seminal vesicle), the wall of the organ may be sufficiently distended to cause the disappearance of the epithelium in places so that the underlying muscles abut directly on the lumen (fig. 6). Similar conditions have been described by Lang (l.c.) in the penis of *Yungia aurantiaca*, Luther (l.c.), in the atrium copulatorium of *Castrada segne*, the bursa copulatrix of *Mesostomidae*, etc.

We thus find in the Turbellaria a complete series of stages in the modification of a normal epithelium to one with insunken nuclei and cell boundaries, not readily demonstrable except by special methods of technique,¹⁰ and similar transition stages occasionally occur in the same species as pointed out by von Graff ('91, p. 6) in the following words:

. . . bei ihnen (*Convoluta sordida* and *paradoxa*) ein und dieselbe Härtungs- und Färbungsmethode (Hämatoxylin z. B.) bald zahlreiche Epithelkerne hervorhebt, bald gar keine oder doch nur sehr wenige Kerne des Epithels tingirt, wenn auch in allen übrigen Theilen bei beiden Individuen die Tinktion eine gleich tadellose wäre.

Von Graff ('99) has called attention to this transition, pointing out the resemblance between the insunken epithelium of Turbellaria and the 'epithelium' of trematodes and cestodes. He says (l.c., p. 42):

Berücksichtigt man dass in der ursprünglichsten Familie (*Geoplaniidae*) . . . ein normales Epithel angetroffen wird, so kann man in den eingesenkten Epithelien überhaupt und speciell in dem der

¹⁰ Not even then, in all cases, fide Böhmig ('90).

Kriechleiste nur einen secundären Charakter erkennen—einen Charakter, der erst in den beiden am weitesten differenzirten Familien der Rhyncodemidae und Bipaliidae auftritt und bei letzterer seinen höchsten Ausbildungsgrad erreicht hat. Hier ergreift der Process der Einsenkung bei manchen Formen, wie *Plac. kewensis*, das gesamte Körperepithel und man könnte sagen dass diese Species und die ihr im Baue des Epithels zunächststehenden Bipaliiden im Begriffe sind, die Epithelform der Trematoden und Cestoden zu acquiriren.

My own observations emphasize this view of von Graff, which has not yet received sufficient notice. I must, however, differ from him in assigning an epithelium to cestodes and trematodes.¹¹ My own view is that we have progressing in the Turbellaria an epithelial transformation leading to the condition in the former groups in which the epithelium has been replaced by a cuticula. I find this process occurring in ontogeny in the vagina and penis of *Taenia serrata*, as I hope to explain more fully in a forthcoming paper.¹²

A similar process has been described by Lönnberg ('91) in the vagina of *Abothrium rugosum* and *Tetrarhynchus tetrabothrius*; he has also pointed out the probable homology between the turbellarian epithelium and the cestode cuticula.¹³

The presence of nuclei in the cuticula of the primitive cestode *Amphilina* (Salensky, '74) and in several trematodes (*Monostomum mutabile*—Braun, '93; *Distomum* sp.—Maclaren, '05; *Cotylogaster*—Nickerson, '02, etc.) suggests that these are forms in which the outer layer has not yet been fully evolved into the cuticula typical of these worms.

The many observations among both trematodes and cestodes of the sloughing of the larval epithelium, this being later replaced by a cuticle formed from underlying tissues (Leuckart, '86, Looss, '92, '93, '94, Pratt, '98), etc., does not, I believe, detract from the soundness of this view, because the homology of the larval epithelium is by no means certain. Until more is known concerning the germ layers of plathelminths, speculation on this latter point is futile.

¹¹ See my discussion of this question elsewhere (Young ('08).

¹² Here however there is apparently no insinking of nuclei into the parenchyma.

¹³ See also Ziegler ('05).

The many variations of the turbellarian epithelium, not only on the surface of the body but also in the digestive and reproductive apparatus, indicate the plasticity of this layer and the probability of the theory outlined above.

LITERATURE CITED

- BÖHMIG, L. 1890 Untersuchungen über rhabdocoele Turbellarien, II, Plagiotomina und Cyliodrostomina, Graff. Zeit. wiss. Zool., Bd. 51, pp. 167-314.
- BRAUN, M. 1893 Trematoda, Bronn's Kl. und Ord. des Tierreichs, Bd. 4, 1 a.
- FUHRMANN, O. 1898 Neue Turbellarien der Bucht von Concarneau. Arch. d'Anat. micros., Bd. 1, pp. 458-80.
- GRAFF, L. VON 1891 Die Organisation der Turbellaria Acoela. Leipzig.
- 1899 Monographie der Turbellarien, II, Tricladida terricola (Landplanarien). Leipzig.
- 1904 Marine Turbellarien Orotavas und der Küsten Europas, I, Einleitung und Acoela. Zeit. wiss. Zool., Bd. 78, pp. 190-244.
- 1905 Idem, II, Rhabdocoela. Zeit. wiss. Zool., Bd. 83, pp. 68-150.
- 1908 Turbellaria, Bronn's Klassen und Ordnungen des Tierreichs, Bd. 4, I c.
- JANDER, R. 1897 Die Epithelverhältnisse des Tricladen Pharynx. Zool. Jahrb. (Anat. und Ont.) Bd. 10, pp. 157-204.
- LANG, A. 1884 Die Polycladen (Seeplanarien) des Golfes von Neapel. Fauna und Flora des Golfes von Neapel . . . herausg. von der Zool. Sta. in Neapel. Leipzig.
- LEUCKART, R. 1886 The parasites of man . . . Translated by William E. Hoyle, Edinburgh and Philadelphia.
- LÖNNBERG, E. 1891 Anatomische Studien über skandinavische Cestoden, I. Kgl. Svenska Vetensk.-Akad. Handlingar, Bd. 24, no. 6.
- 1892 Idem, II. Kgl. Svenska Vetensk.-Akad. Handlingar, Bd. 24, no. 16.
- LOOSS, A. 1892 Über Amphistomum subclavatum und seine Entwicklung. Festschrift Leuckart's, pp. 147-67.
- 1893 Zur Frage nach der Natur des Körperparenchyms bei den Trematoden. Ber. k. sächs. Gesell. Wissenschaften (Math.-phys. Classe), pp. 9-34.
- 1894 Die Distomen unserer Fische und Frösche. Biblioth. Zool., 16, pp. 1-64.

- LUTHER, A. 1904 Die Eumesostominen. Zeit. wiss. Zool., Bd. 77, pp. 1-273.
- MACLAREN, N. 1903 Über die Haut der Trematoden. Zool. Anz., Bd. 26, pp. 516-24.
- NICKERSON, W. S. 1902 Cotylogaster occidentalis. Zool. Jahrb. (Systemat.), Bd. 15, pp. 597-624.
- PRATT, H. S. 1898 A Contribution to the life-history and anatomy of the appendiculate distomes. Zool. Jahrb. (Anat. und Ont.), Bd. 11, pp. 351-88.
- SALENSKY, W. 1874 Über den Bau und die Entwicklungsgeschichte der Amphilina. Zeit. wiss. Zool., Bd. 24, pp. 291-342.
- WOODWORTH, W. M. 1891 Contributions to the morphology of the Turbellaria, I, On the structure of Phagocata gracilis Leidy. Bull. Mus. Comp. Zool. Harvard, vol. 20, no. 1.
- YOUNG, R. T. 1908 The Histogenesis of Cysticercus pisiformis. Zool. Jahrb. (Anat. und Ont.), Bd. 26, pp. 183-254.
- ZIEGLER, H. E. 1905 Das Ectoderm der Plathelminthen. Verh. deutsch. zool. Gesellschaft, 15 Vers.

THE BILATERALITY OF THE PIGEON'S EGG

A STUDY IN EGG ORGANIZATION* FROM THE FIRST GROWTH PERIOD OF THE OOCYTE TO THE BEGINNING OF CLEAVAGE

PART I

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FORTY-SEVEN FIGURES

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I. INTRODUCTION

Whitman in his paper on the development of *Clepsine* ('78), gave the first full and connected account of egg organization; since then much evidence has accumulated to show that many eggs are highly organized before cleavage begins and there are cases in which the origin of this organization has been traced back into the ovarian history of the egg. Thus it is well known that the axis of bilaterality, one of the most fundamental manifestations of organization, appears, in the insect, while the egg is still in the ovary and there is some evidence that the ovarian egg in at least two primitive vertebrates has a bilateral structure. These facts make the relations between the egg and the embryonic axis of certain reptiles and birds very suggestive. The work of Kölliker ('76), Duval ('84) and others established the existence, in the hen's egg, of a fairly definite relation between the embryonic and chalazal axes and similar conditions were found in the pigeon's egg by Patterson ('09). This relation cannot have arisen after the chalazae have been laid down in the oviduct; I have met with only two considerations of the significance of this fact, one in a paper written in 1893 which was found among Professor Whitman's unpublished manuscripts, the other is in Lillie's book on the chick ('08). It has been found that in the pigeon the position of the chalazae is determined before cleavage begins and it follows accordingly that the pigeon egg is bilaterally organized shortly after fertilization. Evidence will here be presented to show that the antero-posterior axis of the pigeon not only appears clearly at the time of fertilization but may also be traced back into the ovarian history at least as far as the youngest oocytes found in the adult ovary.

I am indebted to Professor Whitman for the opportunity of working on this material and for many of the birds that were used. It is difficult for a student of his to express the appreciation he feels of the privilege of working under Professor Whitman. His clear insight into fundamentals, his keen criticism and high ideals, his whole personality made every conference with him an inspiration. It is a pleasure also, to express my obligations to Prof. F.

R. Lillie for his constant interest, advice and criticism. I wish to thank Prof. C. L. Bristol, Dr. J. T. Patterson and the members of this Department for their suggestions and coöperation. Dr. R. R. Bensley has shown me many courtesies in the course of the work; his artist, Mr. A. B. Streedain copied figs. 42 and 43 from the original drawings.

II. MATERIAL AND METHODS

Harper ('04), Blount ('09), and Patterson ('09) have discussed in detail the many advantages the pigeon's egg has for studies on the early stages of development in the bird's egg. The technique employed is new for these eggs and will be discussed briefly.

At the outset the necessity of studying the living material must be emphasized. No conclusions can be based on the form-relations of the younger oocytes or of the blastodiscs that are derived solely from preserved material. Certain distortions are unavoidable, even with the most careful treatment, and they must be controlled by the study and measurement of the living cells. The greatest distortions are introduced in cutting thin paraffin sections, so these have been used only to add structural details and for the illustrations. In the latter case they were used because it is easy to photograph them; they are to be considered merely as corroborative evidence as to the conditions observed in fresh or creasote preparations and free hand sections. Entire ovaries cleared in creasote, dissected and studied under the binocular as solid objects afford an easier and more accurate means of interpreting form-relations than could possibly be obtained from the study of sections, and this method, when properly controlled, is to be highly recommended. In this three-dimensional study of the oocytes the following technique was used: Fix the entire ovary for not more than six hours in a mixture consisting of:

Saturated HgCl_2 in normal salt solution.....	94 cc.
Glacial acetic acid.....	6 cc.
Neutral formol (comm. formaldehyde solution neutralized with MgCO_3).....	6 to 10 cc.

The formol should be added at the time of using and the mixture warmed to the body temperature. As has been said, this fixation only preserves the external form-relations of the oocytes. Cytologically the microscopic picture is not true to life and, as the photographs show, the nucleus is always more or less plasmolyzed. After fixation and washing, the ovary is run up through the alcohols to 95 per cent containing a trace of eosin, then creasote is added very gradually during the course of a day or two and the ovary finally studied in pure creasote. The most important factor in avoiding distortions is the gradual clearing. The method involves little shrinkage and the change in shape is within the probable error in measurement and negligible as may be seen from table 1.

The average shrinkage of twenty oocytes, ranging between 1 and 5 mm., was 4 per cent; in no instance was it greater than 5 per cent. The shrinkage involved in paraffin imbedding and cutting may be judged from the following: an oocyte of 2.5 mm. before fixation, measured 2.4 mm. in alcohol and 2.3 mm. in paraffin. It was cut perpendicular to the long axis into 221 sections 10μ thick. This means a total shrinkage of 11.6 per cent. The effect of the compression in cutting may be judged from figure 27, a photograph of a median section of this egg. The two axes shown were nearly equal before cutting.

To study the blood supply the ovary was injected with a freshly prepared, 5 to 7 per cent solution of Higgins' india ink in physiological salt solution (see Evans, '09). After light staining in eosin it was cleared and studied in creasote.

For cytological control the recent mitochondria methods were used. The most favorable fixative is Benda's 'modified Flemming':

One per cent aqueous chromic acid.....	15 cc.
Two per cent aqueous osmic acid.....	4 cc.
Glacial acetic acid	3 drops

This fixation gives a microscopic picture most nearly resembling the appearance of the living oocytes under the immersion lens; the protoplasm appears as a homogeneous ground substance in which are imbedded granules of various sizes and staining reac-

TABLE 1

FIXATIVE	DIMENSIONS OF OOCYTE IN LIFE	AFTER WASHING	IN 95 PER CENT ALCOHOL	IN CREASOTE
Sublimate				
Acetic (10 per cent)	4.25 x 3.95 x 3.90	4.25 x 3.95 x 3.90	4.25 x 3.90 x 3.90	4.20 x 3.90 x 3.80
Formol (10 per cent)	3.35 x 3.20 x 3.20	3.30 x 3.20 x 3.20	3.2+ x 3.1+ x 3.1+	3.2- x 3.1- x 3.1
Sublimate	1.80	1.7+ x 1.60 x 1.60	1.7+ x 1.60 x 1.60	1.70 x 1.6- x 1.6-
Acetic (7 per cent)	2.80 x 2.7- x 2.70	2.8- x 2.65 x 2.65	2.8- x 2.60 x 2.6+	2.70 x 2.6- x 2.6-
Formol (6 per cent) (1 hour)				

tions. In the study of the blastodiscs however, the important objects are to preserve the form relations and to obtain sharp differentiations in the cytoplasm. The sublimate-acetic mixture above mentioned is best for this purpose. The optimum time of fixation is between forty-five minutes and one and a half hours. The whole yolk is dropped into the warm mixture in such a way that the blastodisc floats down; it remains in this position owing to the weight of the glass pin inserted to mark the orientation. When the blastodisc is to be photographed enough 95 per cent alcohol is added so that the egg just rests on the bottom of the dish. After fixation the egg is washed in repeated changes of 35 per cent and 50 per cent alcohol and hardened in 70 per cent overnight. The next day a pentagonal block is cut out as Blount ('09) and Patterson ('09) direct. Material for sectioning is dehydrated in 95 per cent alcohol, gradually cleared in bergamot oil, and imbedded in 55 to 58° paraffin. Most sections were cut $6\frac{2}{3}$ micra thick and mounted with albumen fixative.

After sublimate fixation the best stain is Bensley's ('11) neutral gentian. The copper-chrom-hematoxylin method also gave good results. Intra vitam staining with Janus green and neutral red aided materially in studying the fresh primordial follicles under the 3 mm. immersion lens. The blastodiscs of mature and fertilized ova were studied and usually drawn in the life. They were kept in the Patterson stage incubator ('09) in a mixture of physiological salt solution and egg albumen, and observed with a Zeiss binocular. Continuous observations could be made by this means of stages between fertilization and the completion of the first cleavage if proper precautions were taken to prevent chilling the egg. For the first three hours and probably for much longer the development is perfectly normal.

In studying the yolk spherules of the younger oocytes the ovary was fixed for twenty-four hours in warm neutral formol (10 to 15 per cent) or in a 15 per cent solution of formol in 2.5 per cent aqueous potassium bichromate. After washing, free hand or frozen sections were made, stained in a saturated solution of Sudan III in 70 per cent alcohol, counterstained with a weak alcoholic solution of methyl green, and mounted in glycerine.

III. THE PROBLEM

Attention has already been called to the relation between the chalazal and embryonic axes in the eggs of the hen and pigeon and the matter is considered in detail in section VI. The general character of the relation in the pigeon is shown in diagram I.

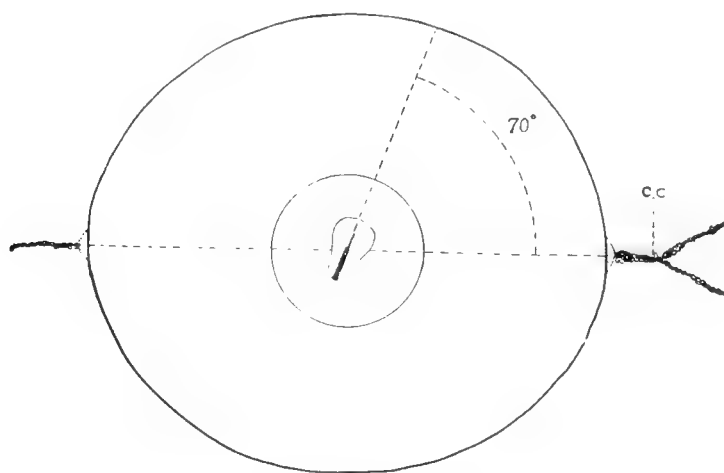


Diagram I A polar view of an incubated pigeon egg ('yolk') showing the relations between the embryonic and long (chalazal) axes. The end of the egg which was directed toward the blunt end of the shell, where the air chamber is always found, is toward the left. The end which passed down the oviduct first, with the 'cloacal' chalaza (*c.c.*) attached, is to the right and the head of the embryo is away from the observer; *c.c.*, cloacal chalaza.

The essential feature is that when the end of the egg opposite the air chamber of the shell is held to the right, the head of the embryo is away from the observer. The chalaza at this end is frequently heavier, double, and more firmly attached than the other; it is in fact the chalaza which is formed first, since the end of the egg to which it is attached goes down the oviduct first. This chalaza may accordingly be called the 'cloacal chalaza' (*c.c.*) for its end of the egg, in the oviduct and in the ovary as well, is nearest the cloaca; the other will be referred to as the 'infundibular chalaza.' It is evident that one end of the chalazal axis is different from the other with reference to the embryo, that is, the chalazal axis is definitely related to the embryonic axis of bilateral symmetry. Furthermore the chalazal axis is the longest axis of the egg. This fact has not been recognized hitherto

although it is almost as apparent in the hen's egg as in the pigeon's.¹ The chalazal, better the 'long' axis, marks the axis of bilaterality of the ovum as a whole; i.e., but one plane, that passing through the long and polar axes divides it symmetrically. The evidence for this is not only that one end of the long axis is definitely related to the embryo, but also that one end is morphologically different from the other as is shown below, p. 289. There are then, two axes of symmetry in the incubated egg, one of the embryo, the other of the mass of food yolk, which, though they do not coincide, are definitely related to one another. Since the chalazae are laid down very soon after the egg enters the oviduct, this relation must also exist at the time of fertilization.

Evidence will be presented to establish the following theses:

1. The bilateral symmetry of the ovum as a whole, manifested by the long axis of oviducal and laid eggs, is present in ovarian eggs at all stages of development from the primordial follicle on. The long axis of ovarian eggs is the same as that of oviducal eggs (p. 294).

2. The antero-posterior axis of the embryo is predetermined in the ovary because the axis of symmetry of the blastodisc of the ovarian egg bears the same relation to the long axis of the entire ovum as does the embryonic axis in the fertilized egg and in the subsequent stages.

It remains to be seen how much farther back in the life history morphological evidences of bilaterality can be found. The evidence at hand points toward the view that bilaterality as well as polarity are inherent characters of the protoplasm and persist from generation to generation.

¹ Patterson's diagram ('09, p. 68) shows that he observed the long axis in the pigeon and Sonnenbrodt ('08) and Riddle give two dimensions in their measurements of oocytes in the hen but neither author attributed any significance to the matter at the time. The relations do not seem to be so constant in the hen's egg as in the pigeon's. Thus, in a series of over one hundred hen's eggs it was found that in almost one-third of the cases the head of the embryo was directed toward the observer when the pointed end of the egg was held to the right. This matter deserves further study, for it involves the question as to whether the end of the hen's egg that is to pass down the oviduct first is predetermined in the ovary as it is in the pigeon.

It should be borne in mind in this discussion that the facts are presented in the order opposite to that in which the analysis was made and so apparently trivial differences in the youngest oocytes fit into a general scheme of development and become quite appreciable during the cell's linear growth of 700 diameters.

IV. THE OVARIAN HISTORY

Most workers on the bird's ovary have divided the ovarian development into more or less clearly defined periods, usually on the basis of the nuclear phenomena alone. The best of these analyses are those of D'Hollander ('04) for the embryonic stages and of Sonnenbrodt ('08) for the subsequent growth periods. The classification that has been made here is based upon the phases of physiological activity of the oocyte as a whole, i.e., the four periods described are distinguished by the different forms in which the cell organization is manifested.

The primordial follicles, the youngest oocytes found in the adult ovary, form the starting point in this discussion. They are in practically the same stage of development as the oldest oocytes of a chick two weeks after hatching or a pigeon several weeks older, and they may be considered as the final stages of the first period of growth after the differentiation of the oocyte as such. This period, in the case of the primordial follicles, involves a slow increase in size from 10 to 80 μ and the accumulation of deutoplasm in the form of lipid spherules. It corresponds to Sonnenbrodt's period V, in which the chromosomes stain deeply and have a characteristic thickened form.

A. The primordial follicle: (oocytes to 0.09 mm.)

The form relations of the primordial follicles can be studied satisfactorily only in the fresh condition and the following description is based primarily on the study of material dissected out of the living ovary in salt solution, usually stained *intra vitam* and examined with a 3 mm. Zeiss apochromat immersion lens. The cover slip was so supported that the oocytes were not subjected

to any pressure. Under these conditions the following relations may be observed: The polar axis is marked by the peripheral position of the germinal vesicle; another axis can invariably be distinguished, namely, one perpendicular to the plane of the polar axis. This axis is also approximately parallel to the surface of the ovary and it is distinctly greater than any other axis (fig. 1). It will be referred to as the 'long axis' and its essential relation is that the polar axis lies in a plane perpendicular to it. It is found, not infrequently, that the oocyte is so oriented that the polar axis is perpendicular to the surface of the ovary as well as to the long axis and that the animal pole is also the attached pole, the vegetative, the free pole (diagram II A). This relation is neither constant nor essential, but there are many reasons for believing that it represents a typical condition.

Under the high powers (2 mm. apo. imm.) the cytoplasm appears as a suspension of various kinds of granules in a homogeneous ground substance. With the aid of vital stains, three kinds of granules can be distinguished. The most obvious are the large deutoplasmic spherules which form a cap between the germinal vesicle and the vegetative pole; this may be called the 'spherule cap' (figs. 1, 9, 11, 13, *sc.*). The periphery of the oocyte is free from these spherules. They are highly refractive in fresh material and appear yellow by reflected light. They stain intensely with Sudan III and are lipoid in character. At the center of the spherule cap, lying close to the germinal vesicle is a finely gran-

Reference letters

<i>bld.</i> , blastodisc	<i>N.A.</i> , nuclear axis
<i>c.c.</i> , cloacal chalaza	<i>o.p.</i> , posterior margin of outer periblast
<i>c.p.</i> , central protoplasm	<i>P.A.</i> , polar axis
<i>c.st.</i> , cloacal end of stigma	<i>p.p.</i> , peripheral protoplasm
<i>E.A.</i> , embryonic axis	<i>s.c.</i> , spherule crescent
<i>g.v.</i> , germinal vesicle	<i>st.</i> , stalk of follicle
<i>L.A.</i> , long axis	<i>s.ov.</i> , surface of ovary
<i>Lat.</i> , latebra	<i>s.z.</i> , spherule zone
	<i>y.n.</i> , yolk nucleus
----- <i>L.A.</i> , long axis	
..... <i>P.A.</i> , polar axis	
..... <i>N.A.</i> and <i>E.A.</i> , nuclear and embryonic axes	

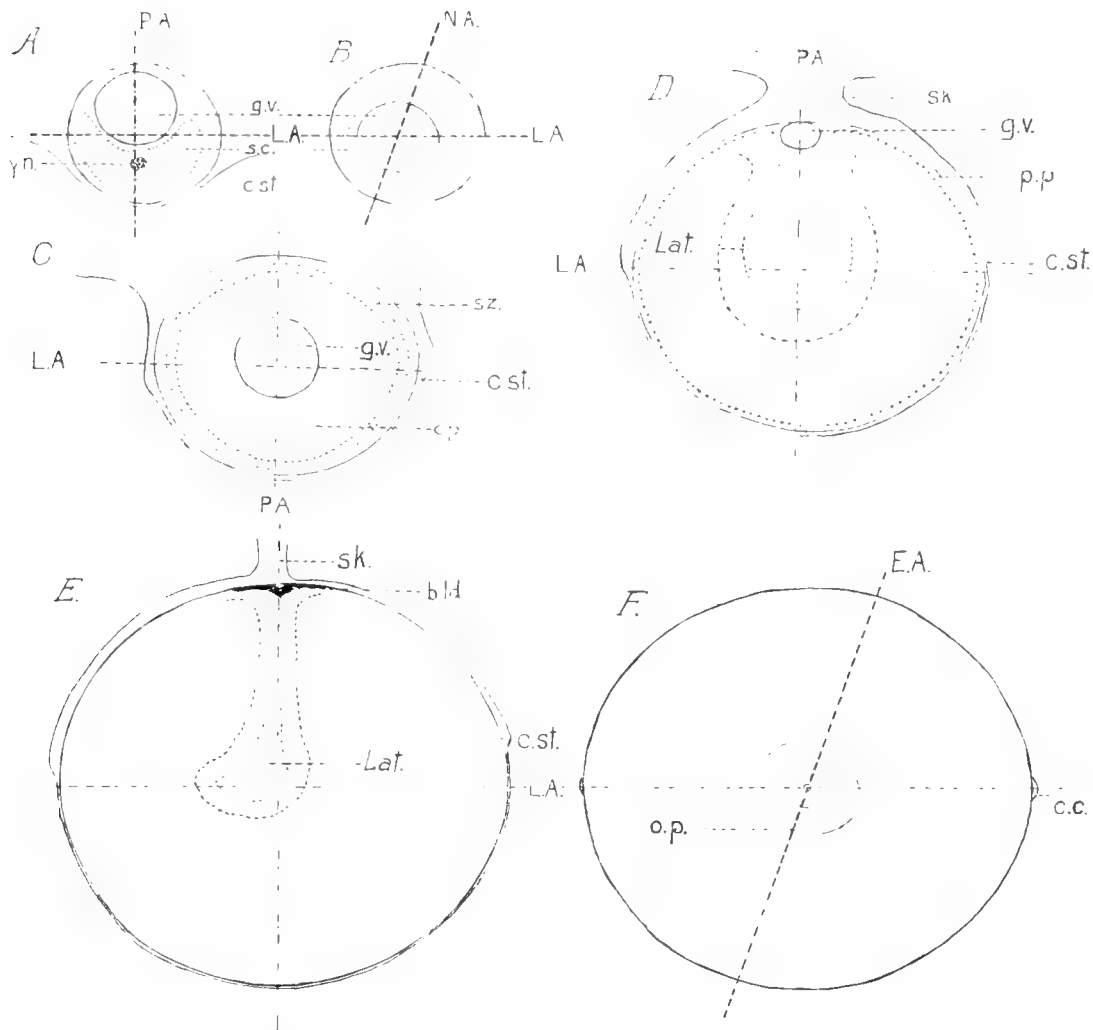


Diagram II Showing the development of the oocyte, and axial relations. *A*, Primordial follicle of 50μ in side view showing the relations of long and polar axes; *B*, the same in polar view, showing the relation of the long (ooplasmic) axis to the nuclear axis; and the relation of the germinal vesicle to the long axis; *C*, Oocyte of 0.6 mm. Beginning of period of differentiation. Side view. The germinal vesicle is near the center of the oocyte but nearest the animal pole and nearer one end of the long axis than the other. The spherule zone lies between the central and peripheral protoplasm. *D*, Oocyte of 3.0 mm. End of period of differentiation; side view. The germinal vesicle is at the periphery; the latebra has appeared and is eccentric in position. The zone of peripheral protoplasm has become narrow. *E*, mature ovarian egg of 20 mm. End of the final growth period. Side view showing the long and polar axes and the eccentricity of the latebra. *F*, Oviducal egg in polar view showing the relation between long axis and the embryonic axis as indicated by the shorter axis of the blastodisc.

ular region (*y.n.*) which stains intensely with neutral red, *intra vitam*; this is the yolk nucleus, part of which at least, is derived from the germinal vesicle and may be considered as cytoplasmic chromatin or 'chromidial substance.' Spherule cap and yolk nucleus, by their position, emphasize the polar axis, which is defined by the eccentricity of the germinal vesicle (fig. 1 and its legend.) The mitochondria form the third kind of granulation and they alone stain with the Janus green. They differ markedly in shape and size from the mitochondria of the follicle cells and of all other cells in the ovary, in being smaller and apparently spherical in shape. Most of the workers on the bird's ovary have either noted or figured the structures that have been mentioned, excepting the mitochondria which are dissolved by all the ordinary fixing fluids. No one however has discussed the question of polarity, doubtless because it seems to be indeterminate (see p. 288).

Two authors have figured primordial follicles in the fresh condition; Waldeyer ('70) and Coste ('47) in his atlas (second 'Poule' plate, fig. 3). This figure is somewhat diagrammatic but it illustrates a constant feature of some interest; viz., the position of the germinal vesicle, nearer one end of the long axis than the other so that the polar axis, instead of bisecting the long axis, cuts it nearer to one end (fig. 1). This means that morphologically the primordial follicle is bilaterally symmetrical; one end of the long axis is different from the other with reference to the germinal vesicle. This long axis is the same as the chalazal axis (p. 294) and it will be remembered that one end of the latter is different from the other with reference to the embryo. The extent of this second eccentricity of the germinal vesicle varies somewhat in different ovaries as may be seen in figure 1 and the photographs, but it is almost invariably distinct in direct side views and polar views.

There is another feature of the primordial follicles which is clear in many ovaries though not in all, but which is very suggestive. The large germinal vesicle is not spherical but elliptical and is so inclined to the long axis of the oocyte that the polar view appears as is shown in figure 1A and diagram IIB. It is obvious that this nucleo-cytoplasmic relation is the same as that between the em-

bryo and the whole ovum in the incubated egg (diagram II *B* and *F*). It is difficult to convince one's self that this relation is of general occurrence, for there are many technical difficulties involved. No confidence can be placed in preserved material and it is not easy to study the fresh oocytes from all angles under the immersion lens; the difference between the two diameters of the germinal vesicle is rarely more than one micron, and it is not apparent except in direct polar views. Still it was possible to observe some oocytes in this way, and in one ovary, where the conditions were especially favorable, it appeared that the angle between the shorter axis of the germinal vesicle and the long axis of the oocyte was relatively constant. This matter is discussed in Section VI, page 299, where it is shown that the same holds true for the angle between the embryonic and chalazal axes. The long axis is definitely related to the embryonic axis of symmetry and it has been shown that it marks the axis of bilaterality of the primordial follicle. If we look upon the elliptical shape of the germinal vesicle as an expression of bilaterality also, we have a basis here in this nucleo-ooplasmic relation of the remarkable relation between the embryo and the ovum as a whole in the incubated egg.

To sum up: Two axes of symmetry can be distinguished in the primordial follicle; (1) The polar axis, which is marked by the eccentric germinal vesicle, the yolk nucleus and the spherule cap. (2) The axis of bilaterality defined by the long axis of the oocyte with the germinal vesicle nearer one end of it. The relation of this bilaterality of the oocyte as a whole to the bilaterality of the embryo is traced in the following sections. (3) There is some evidence to justify the interpretation that at this stage the germinal vesicle has an axis of bilateral symmetry which bears the same relation to the long axis of the ovum as later the embryonic axis bears to this same long axis.

B. The second growth period: (0.09 to 0.4 mm.)

Sonnenbrodt ('08) has emphasized the fact that the primordial follicles of the adult ovary are in a quiescent state; indeed the

only evidences of activity between hatching and maturity are the growth to a maximum of 0.09 mm., and the accumulation of the deutoplasm of the spherule cap. When a primordial follicle begins to grow, however, striking changes occur in the germinal vesicle and the ooplasm. The chromosomes lose their thickened form, become more finely granular and longer, so that they stain more lightly, and many nucleoli are formed apparently at their expense (figs. 10, 13, 14, 15, 16, 18). In the ooplasm more spherules are laid down so that the peripheral zone becomes narrower, (figs. 13 and 17), and there is a marked increase in the yolk nucleus material (chromidial substance?), due largely, as D'Hollander ('04) and others have contended, to a transfusion of chromatin through the nuclear membrane; the striking observations of Munson ('04) are of especial interest in this connection. Figure 14 shows the increase of the yolk nucleus in an oocyte at the beginning of the second growth period. Figure 17 shows the yolk spherules at this stage; it was taken from a free hand section stained with Sudan III. The red stained spherules (black in the photograph) are present throughout the ooplasm except in the narrow peripheral zone and in the region occupied by the yolk nucleus which has increased greatly in amount and is beginning to spread. Figure 15 shows this spreading of the yolk nucleus material clearly. The irregular blocks and bands that may extend into any part of the ooplasm are the 'Balken' of Holl ('90). Figures 16 and 18 show later stages of this process, only one of the numerous 'Balken' appearing in the sections. In both, the individual basophile granules may be seen scattering in all directions; eventually they become evenly distributed through the ooplasm. Occasionally the original center of the yolk nucleus may persist in later stages (fig. 20), but usually the ooplasm now comes to appear homogeneous in paraffin sections. The study of material stained with Sudan shows, however, that yolk spherules are still being laid down at the periphery of oocytes of 0.3 to 0.4 mm. Figure 19 shows an oocyte of 0.3 mm. in which the spherules are confined for the most part to a zone, *sc*, just inside the peripheral protoplasm. This clearing of the central part of the oocyte, defining the central and peripheral protoplasm with the yolk zone between

them, marks the beginning of the period of differentiation (see p. 286).

2. *The origin of the stigma and the follicular blood supply.* The characteristic 'stigma' of the ovarian follicles in the bird has long been known, but so far as I am aware nothing has been published concerning its origin, relations or its exact rôle in ovulation. Since the long axis of the oocyte has not been understood either, no one has noted that the stigma is almost invariably in the long axis.

Mechanical factors seem to play an important part in the differentiation of the stigma and of the closely correlated blood supply of the follicle; at the same time both are definitely related to the bilateral organization of the oocyte.

The stigma does not necessarily arise at a definite stage in the development of the oocyte; it is present in some primordial follicles of $50\ \mu$ and occasionally follicles of $500\ \mu$ are found imbedded in the stroma which show no trace of it. The reason for this is that the essential feature of stigma formation is the intimate association of the follicular epithelium of the oocyte with the peritoneal ('germinal') epithelium, and this association is conditioned by the oocyte's reaching the surface of the ovary. How early in the embryonic history this association of epithelia may take place has yet to be studied, but it is known that neither oocytes nor follicle cells develop directly from the superficial layer of the ovary, 'les cellules indifférentes superficielles' of D'Hollander ('04), so it can hardly come about until the follicle is formed and the oocyte begins to grow. It has already been said that the oocytes are so oriented in the stroma that the long axis is approximately parallel to the surface of the ovary. Accordingly, when the follicle reaches the surface, the fusion first occurs in the great circle of the long axis and an elongate area of fusion is soon formed, the longer diameter of which coincides with the long axis of the oocyte. This appears in figures 3 and 4 where the region free from capillaries indicates roughly the area of stigma fusion. Coincident with the apposition of the two epithelia there is a flattening out of the germinal epithelium. A correlation is established here, between the long axis, itself a manifestation of the egg organization and the other elements of the ovarian follicle,

to form the stigma, which, as will be seen, plays an important part in the orientation of the ovum in the oviduct. Figure 11, taken from a median longitudinal section of an oocyte 83μ in long axis, shows the close approximation of the follicle and epithelial cells; in the whole stigmal area of this oocyte only a few isolated stroma cells were to be found between the two epithelia. The study of many ovaries shows that the stigma is formed accurately in the long axis of the oocyte; the Y-shaped and branched stigmas found in many of the follicles of certain ovaries have their main axis in the long axis of the oocyte and actual deviations from this condition are rare.

In the majority of cases the oocytes come into contact with the germinal epithelium during the second growth period, (0.15 to 0.4 mm.) and the stigma is formed at the free pole. The latter frequently coincides with the vegetative pole of the oocyte, as in all of the figures, but, as has been said, this is not essential for normal development; the polar axis may lie anywhere in a plane perpendicular to the long axis and so be variously related to the surface of the ovary. Nevertheless the condition indicated in diagram 2 is the typical one, for it is the one found normally in mature oocytes whatever may have been the condition in the earlier stages. The factors which bring about the 'typical' conditions in all oocytes that mature will be discussed in the following section (p. 288).

2. *The blood supply of the follicle.* The development of a distinct blood supply, i.e., the differentiation of a theca vasculosa usually begins soon after the formation of the stigma. The capillaries in the cortex of the ovary course about irregularly between the primordial follicles, as may be seen in figure 2. As the oocyte grows, it projects farther and farther from the surface and owing to the outgrowth and anastomosis of fine branches from the pre-existing vessels, the network becomes finer over the bulging surface (figs. 3 and 4). At the same time there is a proliferation of stroma tissue over the whole surface, except in the region of the stigma, where such growth is inhibited as appears in figure 15, which shows a cross section of a stigma (*st.*) Two layers can now be distinguished in the connective tissue follicle: a very

delicate continuous layer, the theca folliculi, closely applied to the follicular epithelium, and an outer loose theca vasculosa which develops hand in hand with the capillary plexus. The theca vasculosa is absent, naturally, in the region of stigma fusion, for here the delicate outgrowths of the capillaries do not penetrate. Figures 5, 7, and 34 show how sharply the stigma is defined in injected material.

The character and development of the blood vessels in the theca vasculosa is illustrated in figures 3 to 7 and 31 to 34, all of which were taken from whole oocytes cleared in creasote. During the second growth period the vascular network comes to invest the entire oocyte except the stigma which extends along the free pole and up toward the attached pole at either end of the long axis, thus dividing the vascular theca into halves which are symmetrically placed with reference to the long axis (figs. 4 *A* and *B*, and 5.) So it comes about that the stigma, which differentiated with reference to the long axis, in turn plays a part in determining the bilateral arrangement of the blood supply. During the succeeding period of differentiation the bilaterality of the blood supply of the follicle is accentuated by the appearance of a median artery and vein on either side, which lie, roughly in the plane of the polar axis, i.e., perpendicular to the long axis (figs. 6 and 7, and the intermediate stages, figs. 31 to 34). The arrangement of the blood vessels, illustrated in figures 7 and 33, is typical of that found in the subsequent stages, although an outer set of vessels, which develops during the final growth period, makes the bilateral arrangement less obvious, as the larger oocyte in figure 37 shows.

The bilaterality of the blood supply is of interest because it plays a part in the preservation of the bilaterality of the ovum as a whole during the final growth period, when the living ooplasm is confined to the region of the blastodisc and to a delicate film of protoplasm over the periphery of the oocyte.

To summarize: The long axis appears as an expression of the bilateral organization of the oocyte. It determines the position of the stigma and together they determine the bilaterality of the blood supply which helps to insure a bilateral deposition of

food yolk. Thus one of the main features of the bilateral structure of the mature ovum is determined. The position of the germinal vesicle nearer to one end of the long axis determines the other, as will be shown below.

C. The period of differentiation: (0.4 to 5 mm.)

As the oocyte grows from three to four-tenths of a millimeter and the yolk spherules are laid down only at the periphery, the germinal vesicle remains relatively stationary in position, so that it comes to lie nearer and nearer to the center of the cell (compare figures 14 to 17, 19 and 20). During the ensuing stages this process is continued as figures 21 to 23 show. Does it ever become quite central in position? This is a matter of theoretical importance and, since the evidence from paraffin sections cannot be relied upon, it has been decided by a study of creasote preparations of entire oocytes and by the use of thick free hand sections. Figure 6 is from an oocyte drawn in creasote and in this, as in all oocytes studied, the germinal vesicle was nearer one pole, the animal pole. This is the only stage at which there could be any question as to the eccentricity of the germinal vesicle and the fact that it is always nearer one pole, defining the polar axis perpendicular to the long axis, completes the evidence that we are dealing throughout development with the same polar axis. If the germinal vesicle migrated about in response to external forces it should be possible to find cases in which it is quite central. Such cases are not found and this agrees with the statements of all recent workers on the bird and reptile ovary.

The appearance of the oocyte during the earlier stages of the period of differentiation is shown in figures 21 to 23. The first, from an oocyte of 0.51 mm. stained with Sudan III, illustrates the position of the sperule zone (*sz*) near the periphery. The next period of growth does not involve the deposition of yolk spherules and so the peripheral protoplasm becomes wider. Somewhat later, in oocytes of about one millimeter, another zone of spherules is laid down and this is the first evidence of the periodicity in yolk formation which characterizes the final growth period (Riddle, '11). In oocytes from 0.5 to 0.8 mm. (figs. 22 and 23),

two ooplasmic zones may be more or less clearly distinguished, the central and peripheral protoplasm of authors. At this time when the germinal vesicle is still near the center of the oocyte, the first definitive yolk granules (white yolk) appear, as the photographs show.

Now as the oocyte continues to grow, the germinal vesicle begins to migrate peripherally along the polar axis to the animal pole (figs. 24 and 25). This is no wandering of the germinal vesicle to the best nourished region of the cell as Sonnebrodt seems inclined to think; the path to the periphery is predetermined, for the polar axis is never changed. The migration begins in oocytes of about 0.9 mm. and the germinal vesicle is usually quite peripheral when they have reached a diameter of 1.5 mm. Shortly after the beginning of the migration very fine yolk spherules appear in the central protoplasm so that they are again present in all parts of the ooplasm except in the peripheral protoplasm.

During this period characteristic changes occur within the nucleus which have been best described by Sonnenbrodt ('08) for the hen. So far as my examination goes, I can confirm his descriptions for the pigeon in most particulars. It may not be out of place to say that in every oocyte I have studied it was possible to demonstrate the chromosomes or their morphological equivalents.

At any given time one finds in an ovary about twelve, rarely more, oocytes from 2 to 5.5 mm. in diameter. The growth at this stage is due chiefly to an increase of the fluid content and a periodic deposition of yolk granules in the central protoplasm, while the peripheral protoplasm grows thinner and thinner (compare figures 27 and 8 from oocytes of 2.5 and 3.2 mm. respectively). In figures 26 and 27 the path of the germinal vesicle to the periphery is marked by a trail of fine reticulum and in the latter the region that surrounded the germinal vesicle during its stay near the center of the oocyte is clearly defined by the smaller yolk granules. This whole flask-shaped area (*lat*) is the anlage of the latebra. Its position was determined by that of the germinal vesicle and accordingly it is near the animal pole and *nearer one end of the*

long axis. This eccentric position of the latebra plays an important part in the orientation of the ovum in the oviduct as will appear below (p. 292).

4. *The zone radiata and the rotation of the oocyte.* One of the reasons why the polarity of the bird's egg has not been understood hitherto, is that in the period now under discussion all possible relations are to be found between the follicle with its long axis and stigma, and the polar axis of the oocyte. In the preceding and the subsequent stages the relations are constant. Thus in the former practically every follicle shows the polar axis perpendicular to the long axis and many have the animal pole coinciding with the attached pole (diagram II, p. 279). During the final growth period also, the conditions shown in diagram 2, *E* are found almost invariably; i.e., the long axis of the oocyte is identical with the stigmal axis of the follicle and both are perpendicular to the polar axis, the animal pole being at the attached pole of the follicle. How does this come about? It might be supposed that only those follicles in which the 'typical' conditions exist, enter upon the final growth period, but this is not so; for the final growth of the oocyte is not dependant upon the relation of the oocyte to its follicle. The facts are these: Oocytes less than a millimeter in diameter usually show the 'typical' relations. After the germinal vesicle has begun to migrate peripherally all possible relations may be found between the oocyte and its follicle, (fig. 7 shows an extreme case). During the final growth period the 'typical' relations are again found, this time almost invariably. The explanation was first suggested by some experiments which showed that, in the later stages, the oocyte as a whole is free to rotate within its follicle. A bird with growing oocytes was tied down on its back for twenty-four hours; when the abdominal cavity was opened it was found that the germinal vesicles occupied the highest points in the follicles viz: the free poles, and yet the structure of the eggs was normal. The polar axis is therefore not influenced by gravity, as some authors have implied; the oocyte as a whole simply orients itself with reference to gravity. In applying these data to the stages in question the following facts must be borne in mind: The oocytes are lying in

the ovary in all possible relations to the direction of the force of gravity and as the germinal vesicle migrates peripherally many of them are subjected to an increasing strain by the force of gravity, since the cell is growing larger and the animal pole is constantly growing specifically lighter than the vegetative pole. If the strain in these oocytes were to be released by the formation of a lymph space, so that they could rotate within their follicles, they would naturally swing into various positions according to their locations in space (fig. 7). At this very time a structure arises which may be interpreted as such a lymph space; it is the zona radiata which is formed apparently from the follicular epithelium. No method of showing that the rotation takes place between the follicular epithelium and the vitelline membrane was found: the evidence rests solely upon the fact that rotation first appears after the zona radiata has been formed, and upon the absence of any other structural condition that would permit rotation. The striations of the zona would, according to this interpretation, be intercellular bridges extending from the follicle cells and they would offer no obstacle to the freedom of rotation.

This freedom of rotation of the oocyte within its follicle is a matter of importance, for whatever may have been the axial relations in the earlier stages, those shown as typical in diagram 2 are eventually established through its agency. As soon as the final growth period is initiated and yolk is accumulated, the follicle becomes large enough to hang down freely into the body cavity; then its highest point is the middle of the attached pole where the blood vessels enter. The oocyte now orients itself along lines of least resistance, the animal pole, i.e., the germinal vesicle with the anlage of the blastodisc come to lie at the attached pole of the follicle and the long axis of oocyte and follicle becomes perpendicular to the polar axis. The laying down of yolk with reference to the polar axis is controlled by the ooplasm, but the follicle, in large measure, determines the long axis, though the peripheral protoplasm may also play a directive rôle. This orientation of the oocyte makes it possible for the original long axis which determined the follicular long axis, to persist during this period when the great bulk of the oocyte is made up of inert

yolk, in the case of those oocytes in which such conditions as are shown in figure 7 existed during the later stages of the period of differentiation. There are many oocytes in which the 'typical' conditions hold throughout the entire development.

D. The final rapid growth period

The ovary of an adult unmated pigeon has normally from six to twelve follicles in the terminal stages of the period of differentiation, from 3 to 5.5 mm. in long axis; never any larger ones. That is to say, the process of rapid yolk secretion which characterizes the final growth period is initiated by the stimulus of mating. The same holds true for birds that have reared young; the stimulus is received when sexual activity is resumed after the young leave the nest. The only theory that will account for all the observed facts is that the initial stimulus is psychic in character; the bird may be mated with another female, with a bird in another cage, or even with her caretaker, and yet lay eggs. This statement is based on Professor Whitman's extended observations in breeding pigeons, and, needless to say, it is easy to tell when a bird is mated from her behavior. Harper ('04, p. 4 ss.)²

Corresponding to the wide experience that pigeons never lay more than two eggs at a sitting, one usually finds in an active ovary two follicles, differing slightly in size, which are distinctly larger than the rest. When the stimulus is received these two begin to grow, one always keeping a few millimeters larger than the other. Occasionally, (in 6.2 per cent of a total of 261 cases), it happens that three follicles mature at the same time, one of them larger than the other two; a condition which is considered on p. 294; it should be noted that an arrangement of follicles in sets in the hen has been observed by Patterson ('10, p. 105). In the course of about eight days the oocyte grows from 5 to 20 mm. in long axis, the definitive size being relatively constant for the eggs of a given bird. The average is about 20 mm., long axis, 18 mm., polar axis, and 18.5 mm. for the third axis perpendicular to these two; but the eggs obtained from one bird averaged

² See also W. Craig, Oviposition induced by the male in pigeons. Jour. Morph., vol. 22, p. 299, 1911.

16.7 by 14.7 by 16.3 mm. The details of this final period of rapid yolk secretion have been described by Loyez ('05-6), Riddle ('11) and others; suffice it to say, that the yolk is laid down concentrically so that the long axis is preserved and the latebra retains its eccentric position. Figure 38 shows the conditions in an oocyte twenty-four hours before ovulation; the end of the long axis which was directed toward the cloaca is toward the right and it is obvious that the latebra is nearer the other, 'infundibular' end. In oviducal eggs the outlines are no longer so clear and often the eccentricity of the latebra is only represented by an extension toward the infundibular end of the long axis as is shown in figure 39 which is the cut median surface of an oviducal egg (see also description of the figures). In laid eggs the softening of the yolk and transfusions make the relations much less distinct than in the earlier stages.

2. *Correlations in the reproductive apparatus.* As has been said usually but two oocytes enter upon the final growth period together. Shortly after the initial stimulus has been received in the ovary, the oviduct becomes highly vascular and increases in size. In one bird that was studied the larger follicle was 9.1 mm., less than one-half the definitive size, the oviduct with its fimbriated infundibulum was about one-fourth the maximal size and both funnel and glandular oviduct were in peristalsis. In another instance, where the larger follicle was 13.2 mm. (in long axis), the second 8.2 mm. the oviduct was almost full size and the active funnel was wrapped about the larger follicle. The correlation of ovarian and oviducal activities is presumably due to the presence of the internal secretion of the ovary in the circulation. It may be said in this connection that the interstitial cells of the ovary show much greater signs of activity in functioning ovaries than they do in ovaries from birds that had not laid for a long time.

The whole reproductive mechanism is delicately balanced and there are interesting physiological problems here still to be worked out. How is it, for example, that the funnel is attracted to the follicle, and that it, eventually, always clasps the larger one, though in the early stages of the final growth period it may, rarely, be found about the smaller? What determines that usually but two follicles mature at a time and that never more than two

eggs are laid? How comes it that yolk secretion, and other factors are so regulated that ovulation is closely correlated with the nuclear maturation phenomena?

To discuss ovulation and the method of orientation it will be necessary first to consider the relation between mature follicle and oviduct. If the reproductive apparatus of a bird be studied two to twenty-four hours before the rupture of the first follicle is due, the following conditions are found: Oviduct and funnel are in active peristalsis, the latter is closely wrapped about the larger follicle, endeavoring so to speak, to swallow it. Under such conditions the follicle is obviously oriented along lines of least resistance, and accordingly its long axis coincides with that of the oviduct and approximately with antero-posterior axis of the bird. One end of the long axis of the follicle is therefore directed posteriorly, toward the cloaca, and this may be termed the cloacal end of the egg. It will be remembered that, in the incubated egg, the cloacal end of the egg is definitely related to the head of the embryo (diagram I, p. 275) and since the antero-posterior axis of the embryo is determined before ovulation, it follows, when the method of orientation and ovulation is taken into account, that this cloacal end of the long axis is predetermined in the ovary. Figure 36 is from a ventral view of a pair of follicles about twenty-four hours before ovulation. In the larger one the stigma appears in the long axis of the follicle, and the long axis extends antero-posteriorly with reference to the bird. The funnel (*inf.*) contracted in preservation and is seen on the left side of the bird.

How comes it that one end of the long axis is found nearer the cloaca? The explanation appears when a mature follicle is removed from the bird and suspended at the center of the animal pole in an albumen solution of the density of that which fills the body cavity at the time of ovulation; it is found that one end of the follicle is heavier than the other, and this is due to the eccentricity of the latebra (fig. 38) whose position nearer the infundibular end of the long axis is due, as will be remembered, to the corresponding position of the germinal vesicle in the early stages. The cloacal end of the ovum, i.e., the end which goes

down the oviduct first, can therefore be traced back to the primordial follicle. The chief factor, then, in the orientation of the follicle is the fact that the egg is heavier at one end of the long axis and, in the normal position of the bird, this end gravitates toward the cloaca. In some cases the position of the ovarian stalk corresponding to that of the latebra may also help in the orientation, but this not a constant feature. The orientation of the follicle in the oviducal axis is due, in the pigeon, primarily to the activity of the funnel; its pressure from without, together with the ever increasing pressure from within, i.e., from the continued yolk secretion, are the main factors in the rupturing of the follicle.

The pressure due to yolk secretion is considerable as may be judged from the way in which the egg bulges out when the rupturing of the follicle begins and also from the observation that the egg is over a millimeter in diameter greater after ovulation than the whole follicle was before.

The funnel can exert considerable pressure, for its wall is muscular and it is attached anteriorly by part of the dorsal oviducal ligament to the left body wall and posteriorly by the ventral ligament to the ventral margin of several coils of the oviduct. Miss Curtis ('10) has clearly brought out these latter relations in the hen and made several illuminating suggestions on ovulation. She describes a 'pocket' formed by the body wall, the left abdominal air-sac and the intestine with its mesentery on the right side, surrounding the ovary. The mouth of this pocket is occupied by the funnel. These relations undoubtedly play a part in the orientation of the follicle and ovulation, and decrease the possibility of the egg escaping into the body cavity. In the pigeon however, I believe that the peristaltic action of the funnel and the yolk secretion are the principal factors in orientation and ovulation, for all these other conditions may be modified by keeping the bird on her back during ovulation and still the ovum succeeds in entering the oviduct.

The orientation may take place in the course of two and a half to three hours, which is the time that elapses between the laying of the first egg and the rupture of the second from the ovary,

assuming that the funnel is inactive while an egg is in the 'uterus.' The evidence for this is that in a dozen or more cases studied in which the first egg was in the uterus, the funnel was not found about the second follicle and no peristaltic movements were observed in it. This is supported by the fact that in the hen, where there are normally several large follicles, Patterson ('10) found the funnel inactive while there was an egg in the uterus. As has been said above, three follicles occasionally mature in the pigeon. In two such cases the following conditions were found. The first egg was laid normally and that night the bird was killed. The second egg was in the oviduct, but in addition a third one was found just ruptured from its follicle and in the base of the funnel. This indicates that the funnel remains active for some time after ovulation, but considering that there is no record of a pigeon having laid more than two eggs at a sitting, it seems probable that after an egg enters the 'uterus,' an anti-peristalsis ejects any other that may be in the oviduct. In one of the cases referred to, the orientation of the follicle in the funnel probably did not take over an hour.

Another important factor in the orientation of the ovum in the oviduct is the way in which the follicle ruptures. The stigma, it will be remembered, extends in the long axis along the free pole of the follicle and so its cloacal end lies at the base of the funnel where this passes over into the glandular part of the oviduct. It will readily be seen that the cloacal end of the stigma is therefore the one part of the follicle where the pressure from within is not balanced by any pressure from without. Now when the various pressures become great enough, the rupture begins at the cloacal end of the stigma (a statement based on the study of over two hundred recently ruptured follicles); usually before the tear has extended along more than 10 mm. of the stigma, the ovum has been squeezed out of the follicle, mainly by the rapidly contracting wall of the latter, and lies at the base of the funnel. The escape of the ovum through an opening one-half its own diameter—I have watched this several times—indicates the elasticity of the extremely delicate vitelline membrane (chorion) and the flexibility of the ovum. In spite of this distortion

the moment the ovum is free it assumes the elongate form it had in the follicle. Eggs newly ruptured from the ovary clearly show the long axis perpendicular to the polar axis and we are dealing with the same long axis in both cases for it is possible to remove a mature follicle from the bird, carefully tear the cloacal end of the stigma, and, under the most favorable conditions for observation, watch the process of ovulation. The long or chalazal axis of the oviducal egg is not, therefore, the result of pressure from the walls of the oviduct, but is the long axis which has persisted from the primordial follicle stage.

As the ovum is entering the glandular portion of the oviduct the walls of the latter attach to the cloacal end a small button which is part of the chalaziferous albumen, and when the whole ovum has entered, the infundibular chalaza is formed at the opposite end of the long axis. Usually the first formed ('cloacal') chalaza is the heavier and it is almost always firmly attached to the egg membrane, while the infundibular chalaza is sometimes represented only by the 'button.' The long axis is now also the 'chalazal axis.'

A word may be said here with reference to the relation of the maturation phenomena to ovulation. No careful study of the maturation spindles, sperm nuclei or pronuclei has here been made, but, so far as I have gone, I have seen nothing except confirmations of Harper's excellent account of these cytological details. The breaking down of the germinal vesicle occurs between six and eight hours before ovulation and usually the processes continue up to the metaphase of the second maturation division, while the oocyte is still within the ovary; they do not proceed any further unless fertilization takes place. The evidence for this is as follows: Three of the eggs obtained at the moment of ovulation were sectioned and the second maturation was found to be just at metaphase; further, eleven eggs taken shortly after ovulation, i.e., eggs found at the beginning of the glandular part of the oviduct were all in the final stages of the second maturation division; finally, it happens occasionally that a follicle fails to rupture within three or four hours of the usual time of ovulation, (8 P.M.) and in these instances also, the equa-

torial plate stage of the second maturation spindle is found. It may be said of the pigeon's egg, then, as of every other well established case in the vertebrates, that the egg does not proceed beyond the metaphase of the second maturation unless it be fertilized. It agrees also with most (perhaps all?) vertebrate eggs in that normally the first polar body is given off in the ovary, and ovulation takes place while the second spindle is in metaphase.

I have no explanation to offer for the definite relation between the breaking down of the germinal vesicle and ovulation. Taking all the data into consideration, however, it would seem that the maturation processes begin soon enough so that they may progress as far as the middle of the second maturation and rest there until the yolk secretion and the other factors have brought about the rupture of the follicle. The latter may take place during the first maturation, judging from the fertilized egg figured and described by Harper, '04, figures 6 and 6a, still I am convinced that the account given above describes the usual course of events, since Harper's is the only similar case that has been found.

V. THE BILATERALITY OF THE BLASTODISC

(Summary of Part II)

A. Origin

The only reference in the literature to the origin of the blastodisc in the bird's egg is, so far as I know, Coste's ('47) surmise that it arises from 'le contenu granuleux' (the spherule cap, p. 277, ss.), but the observations of Agassiz and Clark ('57) on the turtle egg are of value in connection with the conditions described here. It has been shown that the spherule cap of deutoplasmic granules is used up during the period of differentiation of the oocyte (p. 286). The first traces of a blastodisc appear toward the end of this period of development, when the oocyte is about 2 mm. long axis (in life), and when the germinal vesicle has begun to flatten out against the follicular epithelium. Figure 26 shows a longitudinal median section of an oocyte in this stage and figure 28 is from the animal pole of the same oocyte more highly magnified. It will be noted that the reticulum, the spaces of which

represent vacuoles in life, shows a finer meshwork about the germinal vesicle than elsewhere. These smaller vacuoles surround the germinal vesicle (*g. v.*) symmetrically on all sides, except where it adjoins the peripheral protoplasm and from this area the blastodisc is differentiated. The same conditions may be observed in figure 27 in which, however, the germinal vesicle has been distorted by compression in sectioning. The development of the blastodisc symmetrically about the germinal vesicle and the coincident changes within the latter indicate that the former differentiates in close association with the germinal vesicle and lend support to the suggestion made above that the nuclear axis of bilaterality is transmitted to the blastodisc. In figure 27 the deeply staining yolk granules, which are present throughout the cell except in the peripheral protoplasm and the latebra, are small in the neighborhood of the germinal vesicle (*g. v.*); they remain as the characteristic granules of the blastodisc. Throughout development they remain connected with the typical white-yolk spheres by all possible transitional forms and are to be looked upon simply as deutoplasm in a form that can be immediately assimilated by the protoplasm. This 'digested' yolk persists only about the germinal vesicle, characterizing the blastodisc. Fig. 8 is from an oocyte with a clearly defined anlage of the blastodisc surrounding the germinal vesicle, which in life has the form of a biconvex lens. Centrally, and at the peripheral margin, the blastodisc merges with the bed of white yolk, the intermediate zone being the anlage of the central and marginal periblast of Blount ('09), the finely granular region about the germinal vesicle giving rise to the segmental disc (see figs. 35, 40 and 43 for the regions in the mature egg). By this time the peripheral protoplasm has become narrower, less so over the blastodisc than over the rest of the periphery (fig. 29).

Oocytes at the end of the period of differentiation, i.e., in the resting stage before the final rapid growth, have the blastodisc well developed; all the definitive ooplasmic structures are laid down. Fig. 7 shows the blastodisc of such an oocyte in oblique surface view, from which aspect the germinal vesicle appears circular. A median but oblique section of the same oocyte (fig.

29) also shows the blastodisc regions, and in adjoining sections a broad connection can be traced between the central periblast and the latebra. The photograph shows an additional feature: just below the peripheral protoplasm is a layer of deeply staining granules, (*wdg.*) extending from the marginal periblast to the germinal vesicle (*g. v.*). This layer of granules appears wedge shaped in sections of later stages and will be referred to as the 'wedge' (*wdg.*), although actually it forms a broad collar, thicker peripherally, about the germinal vesicle. The granules of the wedge are characterized by being somewhat larger than the neighboring granules and by their strong affinity for basic dyes. At its central margin the wedge is continuous with a layer of basic-staining but much smaller granules which form a thin stratum over the germinal vesicle except at its outer edge where they form a thickened rim. From their subsequent relations these have been termed the polar granules (fig. 29, *p. g.*). Both groups can be traced through the succeeding stages, and during maturation and fertilization they are shifted about by cytoplasmic currents so as to form configurations which are among the clearest evidences of the bilateral organization of the blastodisc. These granules take the violet in the neutral gentian stain as intensely as do the nucleoli and the granulations within the yolk spheres, differing in this respect from the rest of the granules of the blastodisc, as appears clearly in figures 29 and 30, 40 and 41.

B. The blastodisc during maturation and first cleavage

For lack of space at the present time the detailed description of the succeeding stages must be reserved for a later publication. The evidences of bilaterality in these stages are essential for the general thesis here maintained and will be briefly summarized. About two days before ovulation the periblastic zones which characterize the mature egg are established, first posteriorly, then anteriorly. A surface view of a blastodisc in this stage is shown in figure 42. This is the first direct morphological evidence that the blastodisc of the oocyte is bilaterally organized. It shows beyond question that the embryonic axis as such exists in the *ovarian egg* and establishes the validity of the reasoning from the

evidence in the ovarian history which independently led to this conclusion.

After ovulation and fertilization, i.e., between the second maturation and the first cleavage, the whole blastodisc gives evidence of its bilateral organization by a progressive change in shape which can be followed in the living egg. At the time of ovulation, which usually takes place when the second maturation spindle is at metaphase, the segmental disc and periblastic zones are circular, so far as can be seen. After fertilization they gradually become elliptical, so that the antero-posterior diameter is shorter than the right-and-left, as may be seen in figures 35, 36 and 43. A periblastic ring which appears at this time gives further evidence of the bilateral organization of the blastodisc. The most striking manifestation of bilaterality, however, is within the segmental disc itself. The granules forming the 'wedge' are gradually moved from the anterior side of the segmental disc and form a crescent around its posterior margin. This movement can be followed in the living egg and will appear clearly if figures 40 and 41 be compared. The changes that take place between these two stages have been worked out in detail and will be published shortly.

VI. AXIS ANGLES

The long (or chalazal) and embryonic axes

It has been seen that in the incubated egg, two axes of bilaterality can be distinguished:

1. The embryonic axis.
2. The axis of bilaterality of the ovum as a whole, which is defined by: (a) the long axis of the ovum; (b) the position of the latebra nearer one end of the long axis.

The relation between these axes is shown in diagram I, and its development is illustrated in diagram II. The essential feature of this relation is that when a definite end of the long axis is held to the right, the head of the embryo is away from the observer. Hitherto this relation has been described as existing between the embryonic and chalazal axes, but it was shown, (p. 295), that the long axis is the primary one, since it determines

the chalazal axis when the egg is oriented in the funnel. A word should be said here with reference to the chalazae. Normally they are attached to either end of the long axis, but sometimes this attachment is irregular. Patterson ('09, p. 68) found this to be the case in 8 per cent of the eggs; however he included in this category, cases in which only a button is attached to the infundibular end of the egg, and no chalazal thickening appears. There are other cases in which the chalazae are not attached to the ends of the long axis, i.e., in which the long and chalazal axes do not coincide. I observed six such out of 299 eggs, namely 2.2 per cent. Considering the mechanical factors involved in the orientation in the oviduct, this much abnormality is to be expected. Accordingly, the results described here on the relation of embryonic to long axis may be compared with those on its relation to the chalazal axis which are given by the previous workers who overlooked the long axis.

Blount ('09) and Patterson ('09) are the only authors who have made statements with regard to the relation between the embryonic and long axes in the pigeon. The latter author states that the angle between them is 45 degrees in 90 per cent of the cases. No estimate of the probable error was given in the method of measurement that was used, which was to orient the egg under the binocular so that the chalazal axis was parallel to the base line of a square ruled micrometer scale in one ocular, then to note the position of the embryo, and derive the angle. There are several sources of error in such a method and the observations were further hampered by the fact that many of the measurements were made on primitive streak stages. (It should be noted that the angles given by Patterson are the complements of those given here.) The method used in this study was to remove the shell in salt solution, noting the relations of the smaller end of the shell, the heavier chalaza, and the air chamber, for the two former mark the end of the egg which went down the oviduct first and are invariably opposite the air chamber. After it had been noted that the long and chalazal axes coincided, the base of a protractor was laid parallel to them, and without moving the head, a slip of glass 3 mm. wide was moved over the protractor so as to coincide

with the embryonic axis and then the angle was read at the center of the glass slip. The probable error in measuring was found to be from two to three degrees and in the measurements the average of three determinations was taken.

The series of measurements so made showed that there is much greater variation than has been supposed in the pigeon's egg, but that it is not so great as Rabaud ('08) found in the hen's egg (see footnote p. 276). This variation is not due to any change of angle as the embryo develops, for in the eggs of a given bird

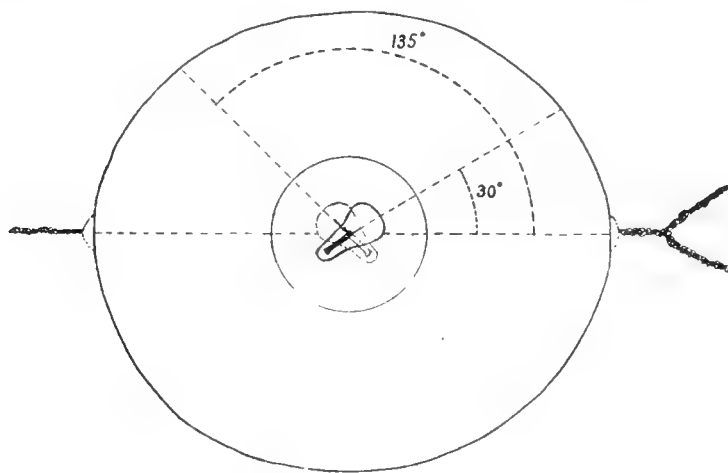


Diagram III A polar view of an incubated egg illustrating the extremes of variation in axis angles in the pigeon. Complete inversion (180 degrees) involves other factors and is not a simple variation, so it is not considered here.

the average of angles measured after thirty-six to forty-eight hours of incubation was the same as after eighteen to thirty-six hours. The same conclusion was reached by Rabaud for the hen's egg after a series of direct observations. Most of the observations recorded here were made on embryos from three to twelve somites. All workers have mentioned a certain amount of variation, even to 180 degrees. In the pigeon only four such cases of complete inversion have come to my attention; one was observed by Dr. Patterson one by Dr. Blount and two by myself; that is, four out of more than six hundred careful observations showed that that end of the long axis which is usually the infundibular one, went down the oviduct first. Aside from these, the extremes of variation observed were 8 degrees and

135 degrees. A curve, (diagram IV) based on all the eggs I studied, viz., 299, taken from eighty-two different birds, shows that these extremes are rare. Four modes appear in the curve, at 50, 70, 80 and 90 degrees. This suggests the most interesting condition that has appeared from this study of axis angles; namely, that the relation between embryonic and long axes is far more constant for the eggs of an individual than for eggs obtained from different birds—a maximum variation of 50 against 127 degrees. Table 2 shows that most of the eggs laid by a given bird have almost identical relations, when one remembers that five degrees is a very slight variation under such conditions as these:

TABLE 2

FEMALE NO. 3		FEMALE NO. 4		FEMALE NO. 2	
Degrees	Eggs	Degrees	Eggs	Degrees	Eggs
90-95	8	35	1	75-80	3
96-100	14	61-65	3	81-85	1
101-105	5	66-70	5	86-90	5
106-110	6	71-75	7	91-95	5
111-115	3	76-80	7	96-100	0
120-125	10	81-85	8	101-105	3
130	1	86-90	5		

It appears that there is one grouping of the eggs of no. 3, about 100 and another about 125 degrees. The fact that it is so exceptional to find a pigeon's egg in which the axial angle is greater than 90 degrees makes the case all the more striking. The record of no. 2 shows that her eggs occasionally had the axis angle greater than 90 degrees; among the eggs of the other eighty birds studied, only twelve of the remaining 287 had angles of 90 degrees or more. The variation in no. 4 is more typical. In the curve, (diagram IV) 66 eggs obtained from the two birds whose axis angles were constantly greater than 90 degrees, are omitted; the dotted line at the right shows the curve with these eggs included.

This constancy of the axis angles in the eggs of a given bird gives further support to the thesis that has been maintained throughout this paper, namely that this relation of axes is determined by factors which are themselves expressions of the bilateral organization of the egg; that is to say, the organization expresses itself in a most constant fashion in the eggs of a given bird. If

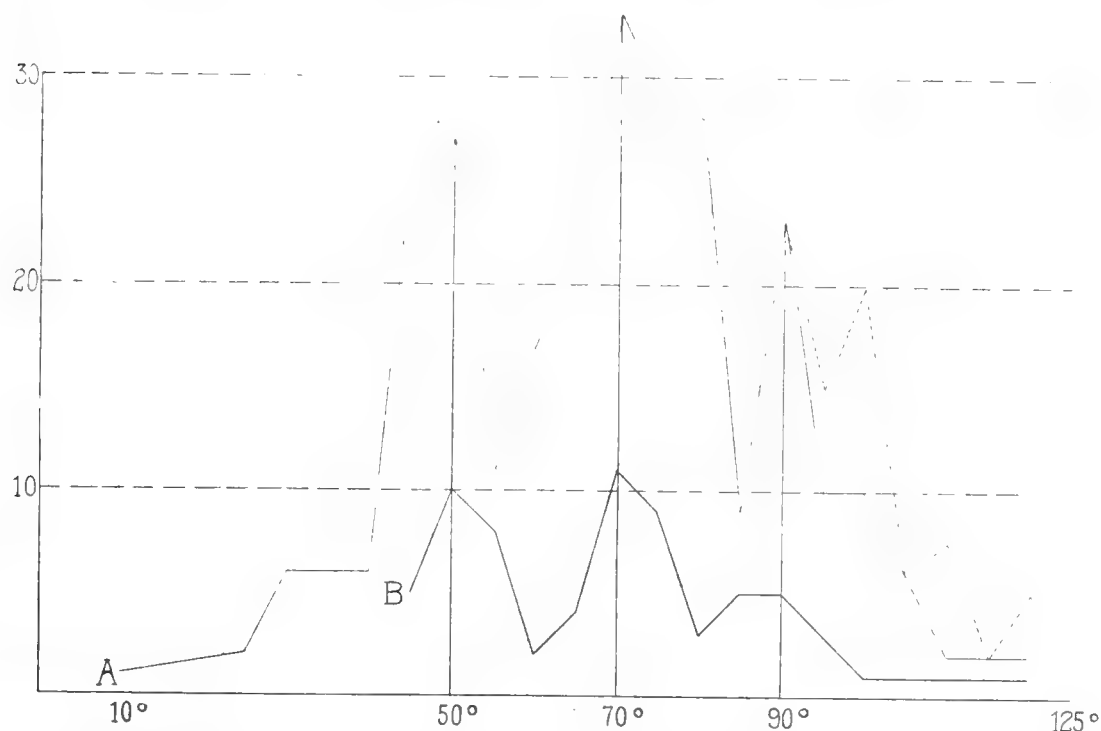


Diagram IV Two curves illustrating the variability in the relation between the embryonic and long axes in the pigeon egg. 'A' was plotted from observations on 299 eggs. They were grouped in 5 degree classes and the number of eggs in a class is plotted on the ordinates, the angles on the abscissae. There are three modes in the 'normal' curve (see text), at 50, 70 and 90 degrees. The broken line at the right represents the angles of the eggs laid by two birds whose norm was above 90 degrees. B (the heavy line) is a curve plotted in a similar way from observations on 59 eggs in stages previous to the third cleavage. The angle measured was between the shorter axis of the blastodisc and the long axis of the ovum. The similarity of the two curves is obvious.

this be true, the nucleo-cytoplasmic relation (diagram II B) in the younger oocytes of a given ovary should correspond to the axis angles in the eggs laid by the same bird. The ovaries of only two of the birds in which a norm had been established, were studied; one was no. 2 mentioned above, the other a bird whose

eggs ranged about 70 degrees. In the ovary of the former many oocytes were found in which the nucleo-cytoplasmic angle was clearly 90 degrees or more, while the majority of the oocytes of the other had an angle less than 90 degrees. This meager evidence may be taken for what it is worth, for obviously the personal factor might find expression through various channels in such a study. Another possible line of evidence in this regard is the study of the axis angles of the eggs laid by the offspring of a bird previously studied. Experiments with this end in view are now in progress. The heavy line B in diagram IV is a curve plotted from the angles observed between the short axis of the blastodisc and the long axis of the whole ovum, in stages previous to the third cleavage. The similarity between the two curves makes it practically certain that the short axis of the blastodisc is the antero-posterior axis, especially when one bears in mind the large body of evidence which goes to show that the anterior end of the embryo is predetermined in the ovary.

Three conclusions may be drawn from these observations on axis angles:

1. The essential feature of the relation between the embryo and ovum as a whole is the fact that the embryo bears a different relation to one end of the long axis than to the other.
2. The eggs of a given bird vary much less as to their relation between embryonic and long axes than do the eggs of different birds.
3. The short axis of the unsegmented blastodisc is identical with the antero-posterior axis of the embryo.

VII. DISCUSSION AND SUMMARY

A. Bilaterality in vertebrate ova

The fact that a fundamental character like bilaterality appears during the ovarian history in so highly specialized a form as the pigeon, suggests the possibility that in the vertebrates as in the insects the antero-posterior axis of the embryo is predetermined in the ovary. The suggestion is strengthened by the striking manifestations of bilaterality which Conklin ('05) describes in

the fertilized eggs of the ascidians. It will be of interest then to consider briefly the evidences and indications of bilateral organization that have been found in vertebrate eggs.

Myxinoids. The ovarian eggs of *Bdellostoma* have been described as being bilaterally symmetrical, their shape corresponding somewhat to that of many insect eggs. Dean ('99) denied this for ovarian or newly laid eggs, but it must be remembered that the question is complicated here, since the eggs are subjected to a great and rapid change in pressure when they are collected. The possibility exists that the antero-posterior axis of *Bdellostoma* may arise in the ovary.

Selachians. The selachian egg presents many striking resemblances to that of the bird, as is to be expected, since these two, together with the reptilian egg are the most extreme types of meroblastic ova. The segmental disc in the selachians, as in the birds, is surrounded by a periblast; in the latter Rückert ('92) found evidences of bilateral symmetry while the egg was still in the ovary. He described in *Torpedo* an extension of the marginal periblast surrounding the blastodisc and projecting down into the yolk mass. This 'Mantel' showed considerable variation in form, but it was found to be constantly deeper and more sharply defined at one side of the disc than the other. In some eggs this structure could be traced from late stages of ovarian eggs, through the fertilization and cleavage stages, and rarely to the time of gastrulation when it appeared as if the deeper side of the 'Mantel' were posterior. Rückert pointed out the significance of this yolk configuration, if it were differentiated along the antero-posterior axis, but he did not consider his evidence as adequate to warrant a definite conclusion. I may say that I have found similar conditions in the eggs of *Raja ocellata*. The variation in these axial relations is to be accounted for as follows: The relations are only expressions of the bilateral organization, not the organization itself; and so the expression varies in different eggs, but is relatively constant for the eggs of a given bird. The relations may be modified by other factors in development but the bilateral organization is nevertheless present; in

other words, these relations simply give evidence that such an organization exists.

It may be then that the selachian and bird's eggs agree not only in that the axis of bilaterality appears in the ovary but also in that the first clear evidence of the bilateral organization of the blastodisc is found in the marginal periblast. This is the only instance that has come to my attention in which definite morphological evidence was found indicating the ovarian origin of the axis of bilateral symmetry in a vertebrate.

Teleosts. Several authors have noted that the blastodisc of teleost eggs is thicker on one side than on the other; Oellacher's ('72) figure 17 indicates that this difference exists before cleavage begins, i.e., that the thickening of the blastodisc after the laying of the egg is more rapid on one side. Agassiz and Whitman, ('85) and Kowalewsky ('86) consider this an antero-posterior differentiation, the thicker being the posterior side, though nothing is said of continuous observations to confirm this. It is quite possible that here as in the Amphibia fertilization initiates the appearance of the axis of bilaterality.

Amphibians. Many workers, notably Roux, Brachet ('05) and Schultze ('99), have shown that the amphibian egg is bilaterally organized previous to cleavage. This organization expresses itself in a definite movement of the superficial pigment granules so that a gray crescent appears at the posterior side of the egg, the center of the crescent marking the position of the blastopore in later stages. It seems unlikely, from what is known of other eggs, that the point of entrance of the spermatozoon determines the axis of bilaterality, as Roux has maintained; bilaterality manifests itself, independently, after the stimulus of fertilization has been received. While the copulation path may determine the plane of the first cleavage, both may be quite independent of the axis of symmetry, as Brachet's work has clearly shown. The arrangement of the different kinds of yolk seems to be radially symmetrical, and how far the bilateral configurations of pigment granules described by Van Bambeke ('76) in the toads' egg are related to the bilaterality of the embryo and how far they are due to the mitotic forces, which here seem to be independent, remains to

be seen when these eggs are sectioned with reference to the gray crescent.

Reptiles. The statement of Will ('93, p. 15) that in the gecko egg the long axis of the embryo is approximately perpendicular to the longest axis of the entire ovum is strong evidence that conditions similiar to those described here exist in that egg. The early appearance of the antero-posterior axis of reptiles is suggested through the analogy of the bird's egg, by the fact that most workers on the early stages have found the blastodisc more or less elliptical in shape.

Birds. Coste ('47), Kölliker ('76), Duval (in his atlas) and Patterson ('10) have figured surface views of the hen's egg in precleavage and early cleavage stages. In every case the segmental disc, the inner periblastic zone and the periblastic ring are shown and Kölliker figures a narrow outer zone about the latter. With one exception these zones are drawn circular, but Patterson's figure 13 shows them elliptical and the shorter axis of the ellipse formed an angle of 90 degrees with the oviducal axis and coincided therefore with the embryonic axis. It is probable that in the hen's egg the bilaterality is not so clearly manifested by the change in shape of the blastodisc as is the case in the pigeon's egg. As has been said above, the orientation in the hen's egg seems to be less constant, but the matter needs further study.

Mammals. The definite orientation of the embryos of various mammals with reference to the chorionic vesicle and the uterus suggest that here too the antero-posterior axis may be traced to an early stage of development.

B. Summary

See diagram II, p. 279

A definite relation exists in the pigeon's egg between the axis of the embryo and the long axis of the ovum.

Both the embryonic and the long axis are present in the ovarian egg, that is, the antero-posterior axis of the pigeon is predetermined in the ovary.

The ovarian history may be divided into four periods in each of which the oocyte exhibits a characteristic organization.

(1) The first growth period, the final stages of which are presented by the youngest oocytes in the adult ovary—the primordial follicles. The primordial follicle has a bilateral structure which can be traced through the ovarian history and which is marked primarily by the fact that the polar axis intersects the long axis nearer to one end.

(2) The¹ second growth period, during which' the bilateral symmetry of the oocyte is impressed upon the connective tissue follicle which in turn plays a part in the preservation of the symmetry of the oocyte as a whole during the subsequent growth. The germinal vesicle comes to lie nearly but not quite at the center of the oocyte, its eccentricity marking the polar axis which is constant throughout oogenesis. Two ooplasmic zones are differentiated: central and peripheral protoplasm.

(3) During the period of differentiation the germinal vesicle migrates peripherally, its path determining the position of the latebra, which as a result of the corresponding position of the germinal vesicle in the preceeding stages, is nearer one end of the ooplasmic long axis. The Anlage of the blastodisc appears about the germinal vesicle. The oocyte becomes free to rotate within its follicle, probably as a result of the formation of the zona radiata.

(4) The final growth period is initiated by the stimulus of mating and in the course of it the great mass of yolk is laid down in such form that the eccentricity of the latebra is preserved.

Data have been obtained on the character of the process of ovulation and the orientation of the egg in the oviduct. The position of the latebra nearer one end of the long axis determines which end of the egg shall pass down the oviduct first and the activity of the infundibulum and other factors orient the follicle so that its long axis coincides with the oviducal axis.

The blastodisc is formed symmetrically about the germinal vesicle and the segmental disc and periblastic zones can be distinguished by the end of the period of differentiation. The embryonic axis first appears unmistakably in the formation of the periblast in the mature ovarian egg. During the final stages of the second maturation, at the time when fertilization has normally

taken place, the antero-posterior axis is expressed by the oval form of the blastodisc and by granule movements in periblast and segmental disc. The most characteristic of the granule configurations is a crescent formed about the posterior side of the segmental disc by certain granules ('the wedge') which are differentiated very early in the history of the blastodisc. These granules are deutoplasmic in character and their arrangement with reference to the bilaterality of the embryo is due to activities within the living substratum, that is, the ground substance.

The essential feature of the relation between the embryo and the long axis of the ovum is the fact that when that end of the ovum which is predetermined in the ovary to pass down the oviduct first, is held to the right, the head of the embryo is directed away from the observer. The actual angle between embryonic and long axes is subject to much greater variation than is generally supposed, but it is relatively constant for the eggs laid by a given bird.

C. Conclusions

If the evidence here offered that the structure of the primordial follicle determines the end of the egg which shall pass down the oviduct first, and that this end is definitely related to the embryonic axis of symmetry be accepted, the conclusion is warranted that the structure of the primordial follicle is a manifestation of the bilateral organization of the oocyte. In other words, the antero-posterior axis of the pigeon is defined at least as early as the stage of the primordial follicle.

The conclusion that the relation between the embryonic axis and the long axis of the entire egg is an expression of the bilateral organization of the ooplasm is also supported by the fact that this relation is much more constant for the eggs of a given bird, than for eggs obtained from different birds.

An explanation of the relation between the long axis of the entire ovum and the axis of the embryo is suggested by a corresponding relation found in many young oocytes between this same long axis and the axis of the germinal vesicle, especially

when it is borne in mind that the blastodisc from which the embryo arises is differentiated under the direct influence of the germinal vesicle.

The fact that in the pigeon the polar axis persists unchanged throughout the growth period of the oocyte, and the fact that there is a polar axis in the earliest stages of the germ cells of all vertebrates which have been described, indicate that the polar axis persists unmodified from generation to generation in the vertebrates and is one of the fundamental features of the organization of the protoplasm. The facts here presented may be taken as evidence that bilaterality appearing as early as it does in development, is likewise an expression of a fundamental character of the protoplasm.

BIBLIOGRAPHY

- AGASSIZ, A., AND WHITMAN, C. O. 1885 The development of osseous fishes. Mem. Mus. Comp. Zool., vol. 14, p. 3.
- AGASSIZ, L., AND CLARK, H. J. 1857 The embryology of the turtles. Contrib. Nat. Hist. of U. S. A., vol. 2, p. 451.
- ALLEN, B. M. 1906 The origin of the sex cells of *Chrysemys*. Anat. Anz., vol. 29, p. 217.
- BALFOUR, F. M. 1878 a A monograph on the development of the elasmobranch fishes. London.
- 1878 b On the structure and development of the vertebrate ovary. Jour. Mic. Sc., vol. 18, p. 383.
- BAMBEKE VAN C. 1876 Recherche sur l'embryologie des Batraciens. Bull. Acad. Roy. Sc. Belgique, Ser. 2, tome, 41, p. 97.
- 1880 Nouvelles recherches sur l'embryologie des Batraciens. Arch. Biol., vol. 1, p. 305.
- 1896 Sur une groupement des granules pigmentaires, etc. (in the toad's egg). Bull. Acad. Roy. Sc. Belgique, Ser. 3, tome 31, p. 29.
- BENSLEY, R. R. 1911 Studies on the pancreas of the guinea pig. Am. Jour. Anat., vol. 12 [technique].
- BLOUNT, MARY 1909 The early development of the pigeon's egg, with especial reference to polyspermy and the origin of the periblast nuclei. Jour. Morph., vol. 20, p. 1.
- BRACHET, A. 1905 Recherches experimentales sur l'oeuf de *Rana fusca*. Arch. Biol., tome 21, p. 103.

- CONKLIN, E. G. 1902 Karyokinesis and cytokinesis in the egg of *Crepidula*. Jour. Acad. Nat. Sciences. Phil. Ser. 2, vol. 12, part 1.
- 1905 The organization and cell-lineage of the ascidian egg. Ibid., Ser. 2, vol. 13, part 1.
- COSTE, M. 1847 Histoire générale et particulière du développement des corps organisés. Tome 1. Paris.
- CURTIS, M. R. 1910 The ligaments of the oviduct of the domestic fowl. Maine Ag. Exp. Station, Bulletin 176.
- DEAN, B. 1899 On the embryology of *Bdellostoma stouti*. Festsch. für v. Kupfer, p. 221.
- D'HOLLANDER, F. 1904 Recherches sur l'oogenèse et sur la structure et la signification du noyau vitellin de Balbiani. Arch. Anat. Mic., tome 7, p. 117.
- DUVAL, M. 1884 La formation du blastoderm dans l'oeuf des oiseaux. Ann. des Sc. Nat. Ser. 6, tome 18, p. I.
- EIGENMANN, C. H. 1892 On the precocious segregation of the sex-cells in *Cymatogaster aggregatus*. Jour. Morph., no. 5, p. 481.
- 1892 Sex differentiation in the viviparous teleost *Cymatogaster*. Arch. f. Entwmech., Bd. 4, p. 125.
- EVANS, H. M. 1909 On the earliest blood vessels in the anterior limb buds of birds and their relation to the primary subclavian artery [technique]. Am. Jour. Anat., vol. 9, p. 281.
- FISCHEL, A. 1903. Entwicklung und Organdifferenzierung. Arch. f. Entwmech. Bd. 15, p. 679.
- GUYER, M. F. 1909 a The spermatogenesis of the domestic guinea. Anat. Anz., vol., 34, p. 502.
- 1909 b The spermatogenesis of the domestic chicken. Anat. Anz., vol. 34, p. 573.
- HARPER, E. H. 1904 The fertilization and early development of the pigeon's egg. Am. Jour. Anat., vol. 3, p. 349.
- HARVEY, B. C. H. 1907 A study of the structure of the gastric glands, etc. [technique]. Am. Jour. Anat., vol. 6, p. 207.
- HERTWIG, O. (Ed.) 1906 Handbuch der vergl. u. exp. Entwicklungsgesch., Bd. 1, no. 1, Jena.
- HOLL, M. 1890 Ueber die Reifung der Eizelle des Huhns. Sitzber. Akad. Wiss. Wien., Bd. 99, p. 311.
- KIONKA, H. 1894 Die Furchung des Hühnereies. Anat. Hefte, Bd. 3, p. 429.
- KÖLLIKER, v., A. 1876 Die Entwicklungsgeschichte des Menschen und der höheren Wirbeltiere. Leipzig.
- KOWALEWSKI, v., M. 1886 Ueber die erste Entwicklungsprozesse der Knochenfische. Zeit. f. Wiss. Zool., Bd. 43, p. 434.

- LILLIE, F. R. 1901 The organization of the egg of *Unio*. *Jour. Morph.*, vol. 17, p. 227.
- 1906 Observations and experiments concerning the elementary phenomena of embryonic development in *Chaetopterus*. *Jour. Exp. Zool.*, vol. 3, p. 153.
- 1908 The development of the chick. New York.
- LOYEZ, M. 1905-6 Recherches sur le développement ovarien des oeufs meroblastique à vitellus nutritif abondant. *Arch. Anat. Mic.*, tome, 8 p. 69.
- MERTENS, H. 1893 Recherches sur la signification du corps vitellin de Balbiani dans l'ovule des mammifères et des oiseaux. *Arch. Biol.*, tome 13, p. 389.
- MORGAN, T. H. 1909 A biological and cytological study of sex determination in *Phylloxera*s and *Aphids*. *Journ. Exp. Zool.*, vol. 7, p. 239.
- MUNSON, J. P. 1904 Researches on the oogenesis of the tortoise. *Am. Jour. Anat.*, vol. 3, p. 311.
- NEWMAN, H. H., AND PATTERSON, J. T. 1909 A case of normal identical quadruplets in the nine-banded armadillo and its bearing on the problems of identical twins and of sex determination. *Biol. Bull.*, vol. 17, p. 181.
- NICOLAS, A. 1900 Recherches sur l'embryologie des reptiles. *Arch. Anat. Mic.*, Tome 3, p. 457.
- OELLACHER, J. 1872 Beiträge zur Entwicklungsgeschichte der Knochenfische nach Beobachtungen am Bachforellenei. *Zeit. f. Wiss. Zool.*, Bd. 22, p. 373.
- OPPEL, A. 1892 Die Befruchtung des Reptilieneies. *Arch. f. mikr. Anat.*, Bd. 39, p. 215.
- PATTERSON, J. T. 1909 Gastrulation in the pigeon's egg. *Jour. Morph.*, vol. 20, p. 65.
- 1910 Studies on the early development of the hen's egg. Part 1. *Jour. Morph.*, vol. 21, p. 101.
- RABAUD, E. 1908 La position et l'orientation de l'embryon de poule sur le jaune. *Ar. Zool. Exp. et Gen. (Notes et Revue)*, tome, 9, p. 1.
- RIDDLE, O. 1911 White and yellow yolk in vertebrate ova. *Jour. Morph.*, vol. 22, p. 455.
- RÜCKERT, J. 1892 Die Entwicklungsgeschichte des Ovarialeies bei *Selachiern*. *Anat. Anz.*, vol. 7, p. 107.
- 1899 Die erste Entwicklung des *Selachiereies*. *Festsch. für v. Kupfer*, p. 581.
- SCHULZE, O. 1899 Ueber das erste Auftreten der bilateralen Symmetrie im Verlaufe der Entwicklung. *Arch. f. mikr. Anat.* Bd. 55, p. 171.

- SOMER DE, E. 1905 Les premiers stades de la vitellogenèse dans l'ovule de la poule. Ann. Soc. Med. de Gand., Tome 85, pp. 55-62.
- SONNENBRODT, 1908 Die Wachstumsperiode der Oocyte des Huhns. Arch. f. mikr. Anat., Bd. 72, p. 415.
- TODARO, F. 1895 Beobachtungen und Betrachtungen über die Furchung des Eies und die Bildung der Keimblätter bei *Seps chalcides*. Untersuch. z. Naturlehre. Bd. 15, p. 520.
- VAN DER STRICHT, O. 1902 Le noyau vitellin de Balbiani et les pseudochromosomes chez les oiseaux. Verh. d. Anat. Ges., 16 Vers., p. 168.
- WALDEYER, 1870 Eierstock und Ei. Leipzig.
- WHITMAN, C. O. 1878 The embryology of *Clepsine*. Quart. Jour. Micr. Sc., vol. 18, pp. 215.
- 1887 The kinetic phenomena of the egg during maturation and fecundation. Jour. Morph. vol., 1, p. 227.
- 1893 The inadequacy of the cell theory of development. Biol. Lectures, Wood's Hole. Boston, 1894.
- WILL, L. 1893 Beiträge zur Entwicklungsgeschichte der Reptilien. (Gecko). Zool. Jahrb., vol. 6, p. 1.
- WILSON, E. B. 1903 Experiments on cleavage and localization in the Nemertine egg. Arch. f. Entwmech., vol. 16, p. 411.
- 1904 Experimental studies on germinal localization. Jour. Exp. Zool., vol. 1, p. 1.
- WOODS, F. A. 1902 The origin and migration of the germ cells in *Acanthias*. Am. Jour. Anat., vol. 1, p. 307

REFERENCE LETTERS

<i>a.o.</i> , anterior margin of outer periblast	<i>o.p.</i> , posterior margin of outer periblast
<i>bld.</i> , blastodisc	
<i>bz.</i> , boundary zone of central protoplasm	<i>pbl.</i> , periblast
<i>cap.</i> , capillary	<i>pbl.r.</i> , periblastic ring
<i>cl.</i> , cloaca	<i>p.g.</i> , polar granules
<i>f.e.</i> , follicular epithelium	<i>p.r.</i> , polar ring
<i>g.v.</i> , germinal vesicle	<i>p.p.</i> , peripheral protoplasm
<i>inf.</i> , infundibulum	<i>s.c.</i> , spherule crescent
<i>i.pbl.</i> , inner periblastic zone	<i>s.d.</i> , segmental disc
<i>mg.dc.</i> , margin of segmental disc	<i>st.</i> , stigma
<i>lat.</i> , latebra	<i>s.z.</i> , spherule zone
<i>m.a.</i> , median artery	<i>ut.</i> , 'uterus'
<i>m.v.</i> , median vessel	<i>wdg.</i> , wedge
<i>o.a.</i> , anterior margin of outer periblast	<i>y.n.</i> , yolk nucleus

PLATE 1

EXPLANATION OF FIGURES

1 Camera drawings of living primordial follicles in median optical section; Zeiss 3 mm. apoch. imm. objective, ocular 6, 480 diameters. (In polar views the germinal vesicle is projected into the median plane.) The conditions illustrated in diagram 2 are shown here; in each case the long axis was parallel to the surface of the ovary and the germinal vesicle was nearer one end of it. *A* and *B* are from ovarian cortex studied in warm salt solution. *A*. Oocyte, $57 \times 43\mu$, polar view, showing the nucleo-ooplasmic relation. The follicular epithelium (*f.e.*) surrounds the oocyte. *B*. Oocyte, $70 \times 63\mu$, side view. The clear region central to the germinal vesicle indicates the position of the yolk nucleus, which lies accurately in the polar axis. *C*, *D* and *E* are from an ovary injected intra vitam with neutral red and Janus green. The yolk nucleus granules were stained deep red and appear solid black in the figure. The mitochondria are not represented but they appeared as very minute granules arranged in fine strings. In this ovary all the primordial follicles had the long axis relatively much greater than the other two and the germinal vesicle was markedly nearer one end of the long axis. On the other hand the difference between the two axes of the germinal vesicle is hardly noticeable. *C* and *E* show a condition that is not uncommon, namely that the polar axis does not bisect the yolk nucleus (*y.n.*) and as is usually the case there are more spherules at one end of the long axis. *C*. Oocyte, $67 \times 52\mu$ in side view showing the granules of the yolk nucleus (*y.n.*). *D*. Oocyte, $78 \times 58\mu$ polar view; *g.v.*, germinal vesicle; *f.e.*, follicular epithelium; *s.c.*, spherule crescent. *E*. Oocyte, $75 \times 53\mu$ in polar view; *y.n.*, yolk nucleus.

2 to 7 Camera drawings illustrating the development of the follicular blood vessels. From the outset the arrangement is bilateral with reference to the long axis. All magnified 100 diameters. Zeiss 16.0, oc. 6. Studied and drawn in creasote as solid objects.

2 Blood vessels of the cortex of the ovary around the primordial follicles. Portion of an adult ovary injected with india ink, fixed in sublimate-acetic-formol, cleared and drawn in creasote. The heavy circles represent the follicular epithelium of the oocytes, (*f.e.*) the fine wavy lines the walls of the capillaries, (*cap.*) and the dotted lines, the germinal vesicles (*g.v.*) which are plasmolyzed.

3 Oocytes of 232μ at the beginning of the second growth period viewed from the free (stigmatal) pole. The capillary network is spreading over the free hemisphere; the stigmatal area is still broad, but its longer dimension coincides with the long axis of the oocyte.

4 Oocyte of 244μ . *A*, side view; *B*, polar view. The vascular network is spreading symmetrically with reference to the long axis, on either side of the oocyte. Other relations as in fig. 3; stigmatal area (*st*).

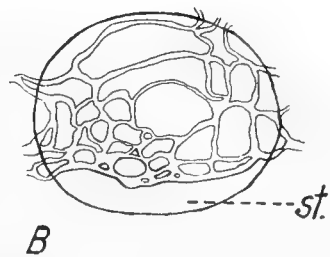
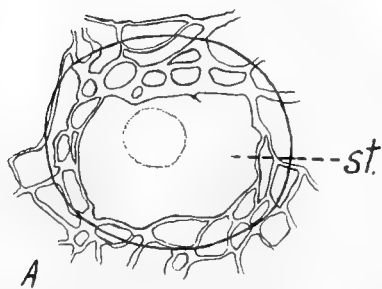
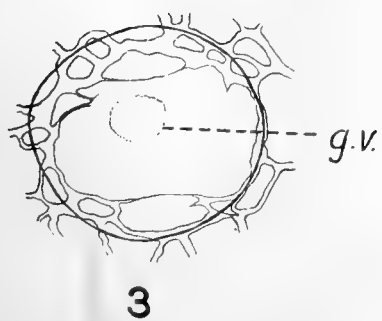
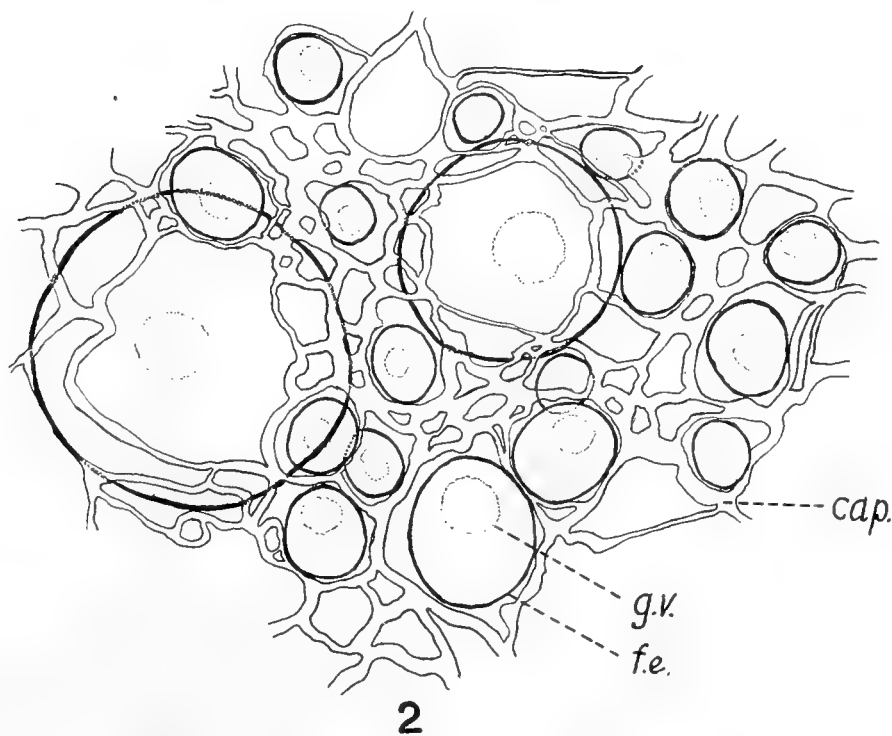
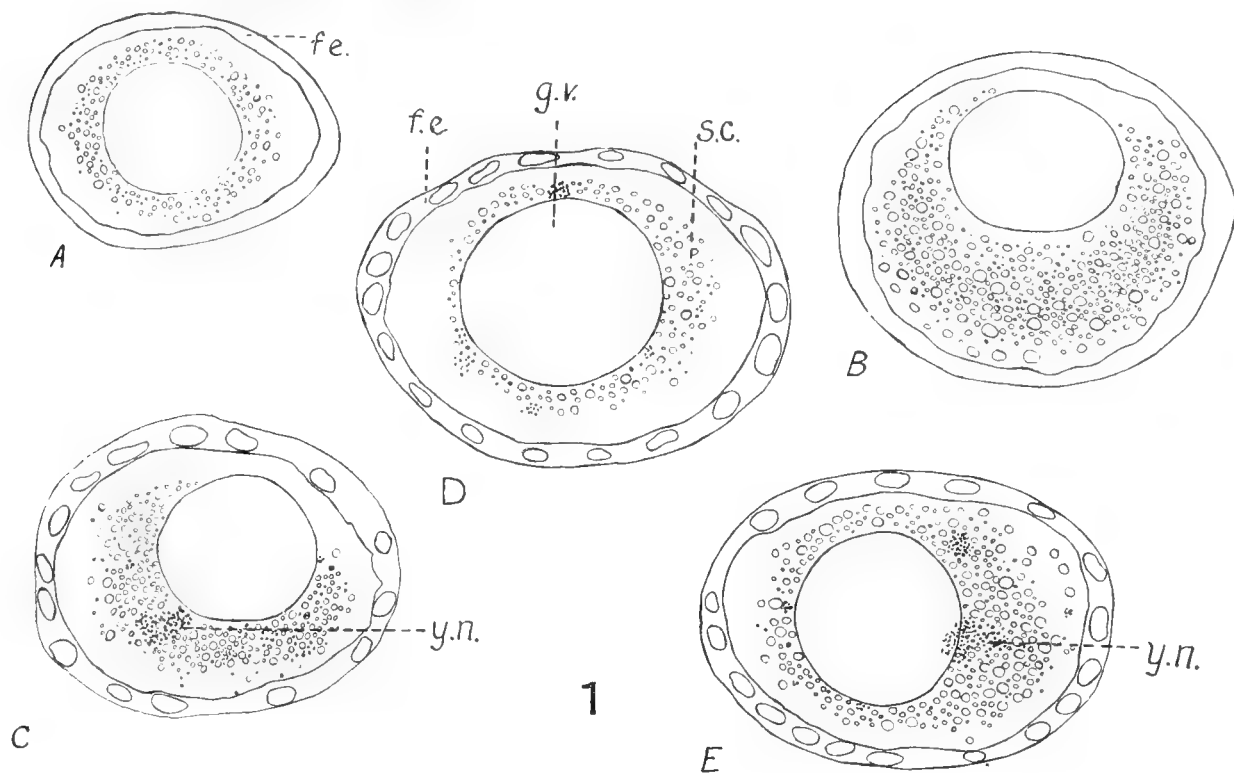


PLATE 2

EXPLANATION OF FIGURES

5 Oocyte, $400 \times 376\mu$, at the beginning of the period of differentiation; oblique view from the stigmal pole. The vascular network completely invests the oocyte except in the region of the stigma (*st.*) which extends in the long axis. A direct view in the polar axis showed the germinal vesicle near, but not quite at, the center of the long axis.

6 Oocyte, $902 \times 829\mu$. Period of differentiation. Side view showing the differentiation of the median vessel (*m.v.*). Note the germinal vesicle (*g.v.*) almost central in position but slightly nearer to the animal pole, which is up, and to the infundibular end of the long axis, to the left.

7 Oocyte, 5.47×5.14 mm. magnified 15 diameters, at the end of the period of differentiation, showing the stigma (*st.*) in the long axis, and the median artery in somewhat oblique side view. The oocyte is free to rotate within the follicle during this period and the germinal vesicle (*g.v.*) surrounded by segmental disc and periblast (*pbl.*) lies at one end of the long axis. This is an extreme case of rotation; usually the rotation is around the long axis. Fig. 34 shows a stigmal view of this oocyte.

8 Animal pole of an oocyte of 3.2 mm. in median longitudinal section, $\times 100$ diameters, Zeiss 16.0, oc. 6. An early stage in the formation of the blastodisc. The germinal vesicle (*g.v.*) is surrounded by the fine granules of the segmental disc, and this in turn by the periblast (*pbl.*). The peripheral protoplasm (*p.p.*) is now a narrow zone. At the center of the germinal vesicle is the group of chromosomes and nucleoli.

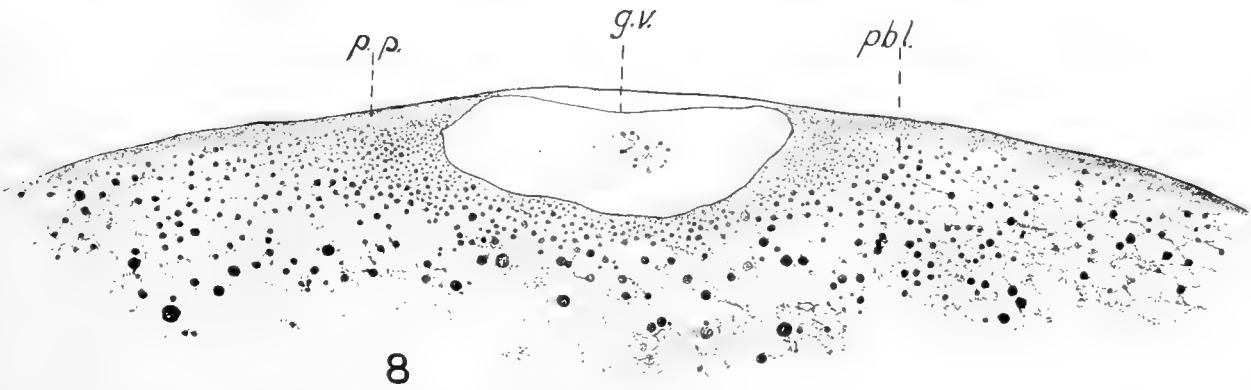
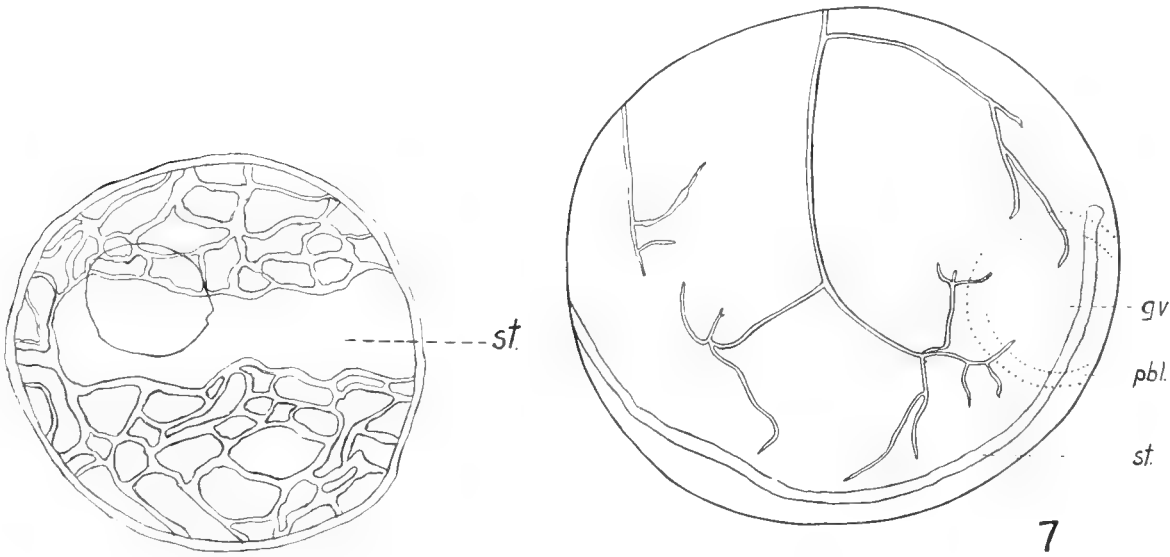
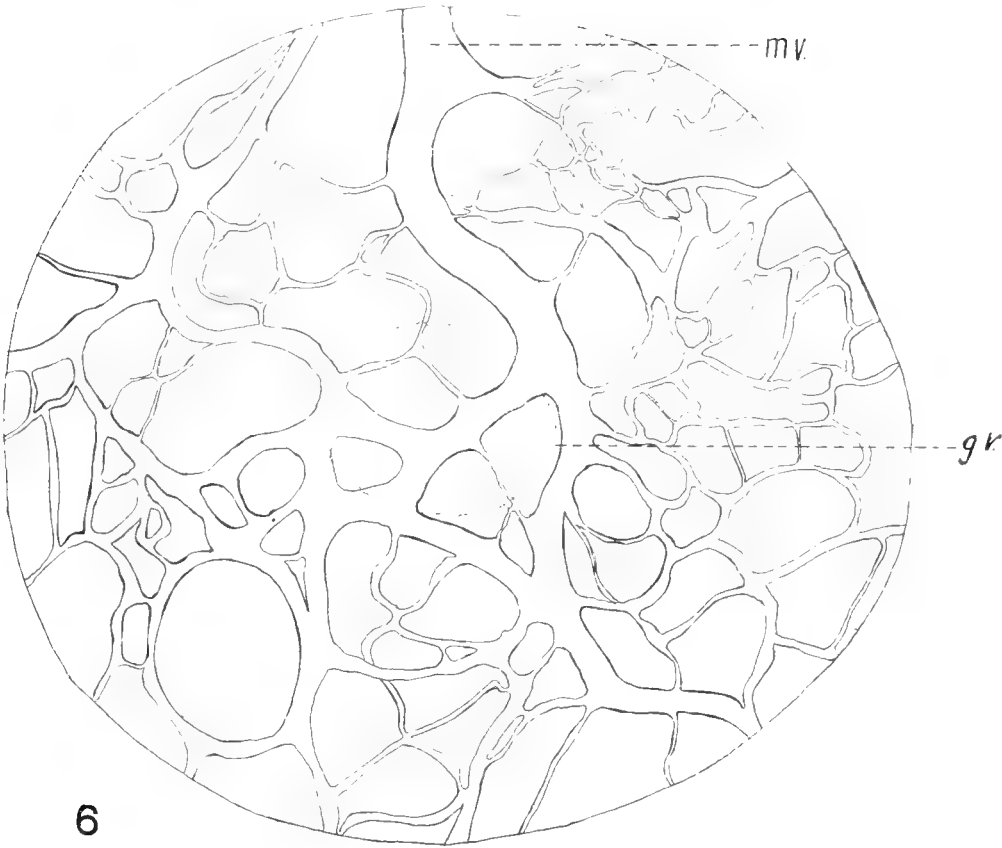


PLATE 3

EXPLANATION OF FIGURES

All the figures except 17 are from photographs of sections of ovaries fixed in sublimate-acetic-formol mixtures, cut 10μ thick in paraffin and stained either with neutral gentian or with neutral safranin-säure violett. All were taken with a Zeiss 4 mm. apochromat, ocular 4 and magnified 350 D. The germinal vesicle is plasmolyzed more or less in every case. In each oocyte the 'typical' relations of diagram 2 appear but the critical evidence for these conclusions was obtained from fresh and creasote preparations and from free-hand sections. The measurements are taken from the sections; the dimensions in life are estimated from 5 to 10 per cent greater.

9 Primordial follicle, $52 \times 44\mu$, cut in the long and polar axes. *y.n.*, yolk nucleus, surrounded by the spherules which appear as vacuoles.

10 Primordial follicle, $78 \times 62\mu$, Horizontal section cut perpendicular to the polar axis, near the animal pole. Note the germinal vesicle nearer one end of the long axis.

11 Primordial follicle, $83 \times 60\mu$, cut in long and polar axes and showing the stigma (*st.*) already formed in the long axis.

12 Primordial follicle, $88 \times 72\mu$, imbedded in the stroma so that the stigma has not yet formed. Cut in long and polar axes.

13 Primordial follicle, $95 \times 83\mu$ cut in long and polar axes showing the relations of germinal vesicle (*g.v.*), yolk nucleus (*y.n.*) and spherule crescent (*s.c.*); *f.e.* follicular epithelium.

14 Oocyte at the beginning of the second growth period, $103 \times 100\mu$, showing the yolk nucleus material increasing in amount.

15 Oocyte 277μ in long axis; second growth period. Cut in the polar axis, transversely to the long axis, showing the stigma (*st.*) in cross section and the increase of the spherules (*s.c.*) The germinal vesicle is still peripheral and the substance of the yolk nucleus (*y.n.*) has increased greatly in amount and is spreading irregularly through the ooplasm.

16 Oocyte, $203 \times 166\mu$; second growth period. Cut in the long and obliquely to the polar axis. A later stage than fig. 15; from another ovary. The germinal vesicle is no longer peripheral and the chromidial substance is diffusing through the ooplasm.

17 Oocyte, $198 \times 162\mu$, cut in the long and polar axes. Photographed from a free hand section of an ovary fixed in formol-bichromate and stained with Sudan III. The lipoid spherules, (*s.c.*) appear black, the lighter central region is the yolk nucleus which is enlarging. The peripheral protoplasm (*p.p.*) is free from spherules.

18 Oocyte $280 \times 255\mu$; magnified 325 diameters and cut almost perpendicular to the polar axis. The section passes through the center of the germinal vesicle, which is distinctly nearer to one end of the long axis. A cross section of one of the 'Balken' appears at *y.n.* and shows clearly the diffusion of the chromidial (?) granules.

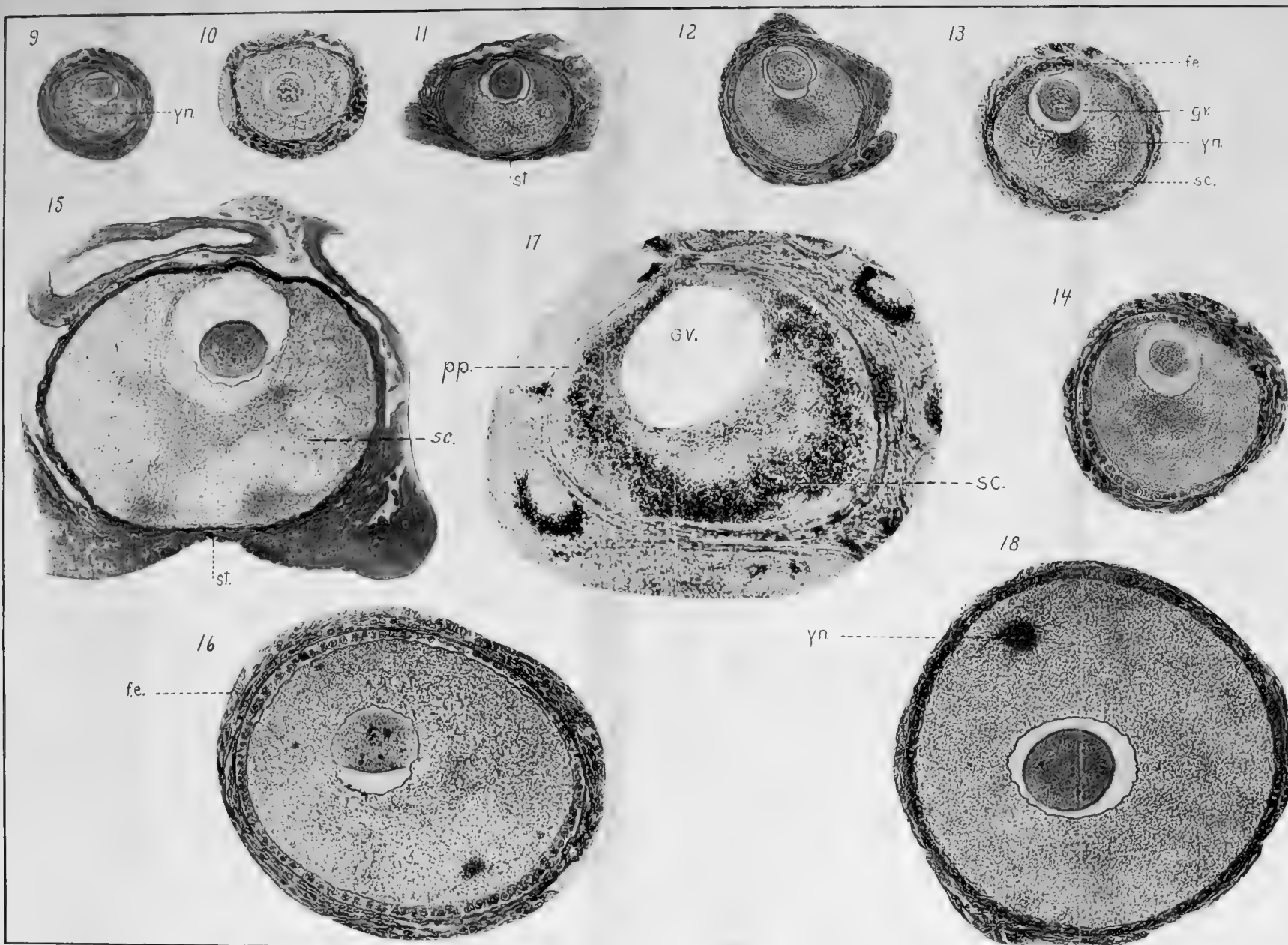


PLATE 4

EXPLANATION OF FIGURES

Photographs from the same series of sections used for plate 3.

19 Oocyte, $293 \times 270\mu$, cut in the long and polar axes. Zeiss 4 mm., oc. 4, reduced to $\times 280$. From a free hand section stained with Sudan III. The spherules are disappearing from the central protoplasm and one can begin to distinguish a spherule zone (*s.c.*) within the peripheral protoplasm (*p.p.*). The size relations between germinal vesicle (*g.v.*), and ooplasm are noteworthy in comparison with the oocytes sectioned in paraffin e.g., fig. 20.

20 Oocyte, $354 \times 324\mu$; magnification as in fig. 19. End of second growth period. Cut in long and polar axes. The persistence of the center of the yolk nucleus (*y.n.*), at this stage is a condition found only occasionally.

Period of differentiation.

Figs. 21 to 25 were taken with a Zeiss 8 mm. apochromat, oc. 2, and reduced to $\times 80$.

21 Oocyte, $512 \times 400\mu$, cut near the animal pole. Photographed from a frozen section, 50μ thick, stained with Sudan. At this stage the spherules form a zone, (*s.z.*), near the periphery, but with a higher power the clear peripheral protoplasm can be distinguished. The alveolar appearance is an artefact due to freezing. Only the tip of the germinal vesicle (*g.v.*) appears.

22 Oocyte, $762 \times 710\mu$, cut perpendicular to the polar axis, showing the germinal vesicle nearer one end (left) of the long axis. The central and peripheral protoplasm (*p.p.*) can be distinguished with the spherule zone between them.

23 Oocyte, $786 \times 762\mu$, cut in the long and polar axes, in about the same stage as fig. 6. At the right the section passes to one side of the stigma (*st.*). The spherule zone (*s.z.*) can be seen and in addition many large definitive yolk granules have appeared throughout the ooplasm.

24 Oocyte, 1.06×0.89 mm., cut in long and polar axes. The germinal vesicle has migrated peripherally out of the central protoplasm, the boundary of which is marked *bz.*

25 Oocyte, 1.17 in long axis, cut in the polar axis and obliquely to the long axis. The photograph was taken from a section nearer to one end of the long axis. The central and peripheral protoplasm and a single spherule zone may be distinguished (more clearly on the right). The definitive yolk granules are especially numerous peripherally. *b.z.*, boundary of the central protoplasm.

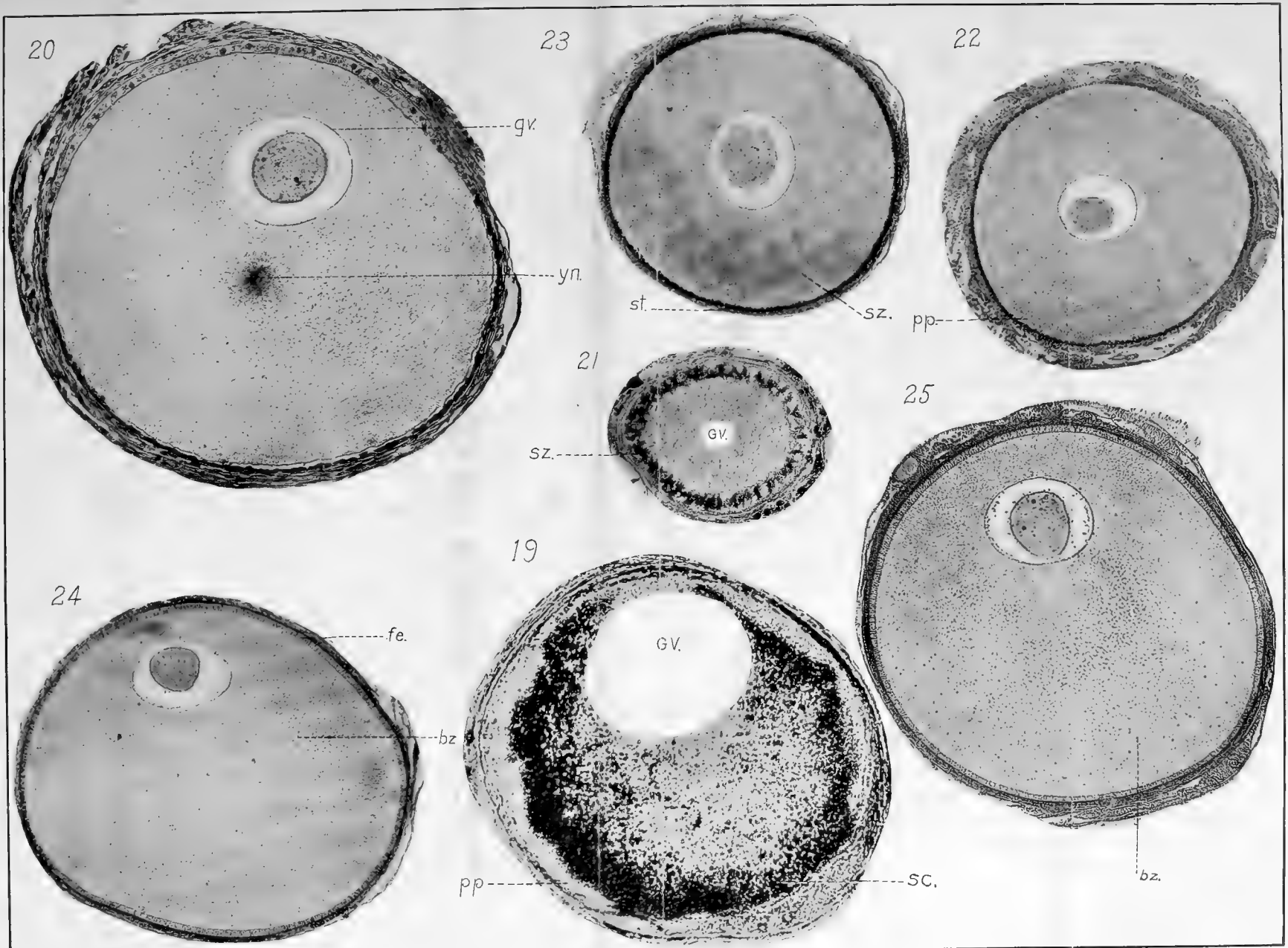


PLATE 5

EXPLANATION OF FIGURES

26 Oocyte, 1.74 x 1.44 mm., cut approximately in the long and polar axes, but much distorted in sectioning. The germinal vesicle is peripheral. The location of the future latebra is indicated by the finer meshwork at the center of the oocyte. Taken with a one-half inch B. and L. objective, $\times 40$. *p.p.*, peripheral protoplasm.

27 Oocyte, 2.5 mm. in long axis (life), $\times 40$, Leitz obj. no. 2. Cut in polar axis, perpendicular to the long axis and compressed from right to left in cutting. The follicular epithelium has shrunk somewhat from the theca folliculi, and there is a shrinkage tear near the germinal vesicle. The region of the latebra (*lat.*) is clearly defined at the center and the layers of yolk granules indicate the rhythmical formation of yolk. The stigma is shown in cross section at *st.*

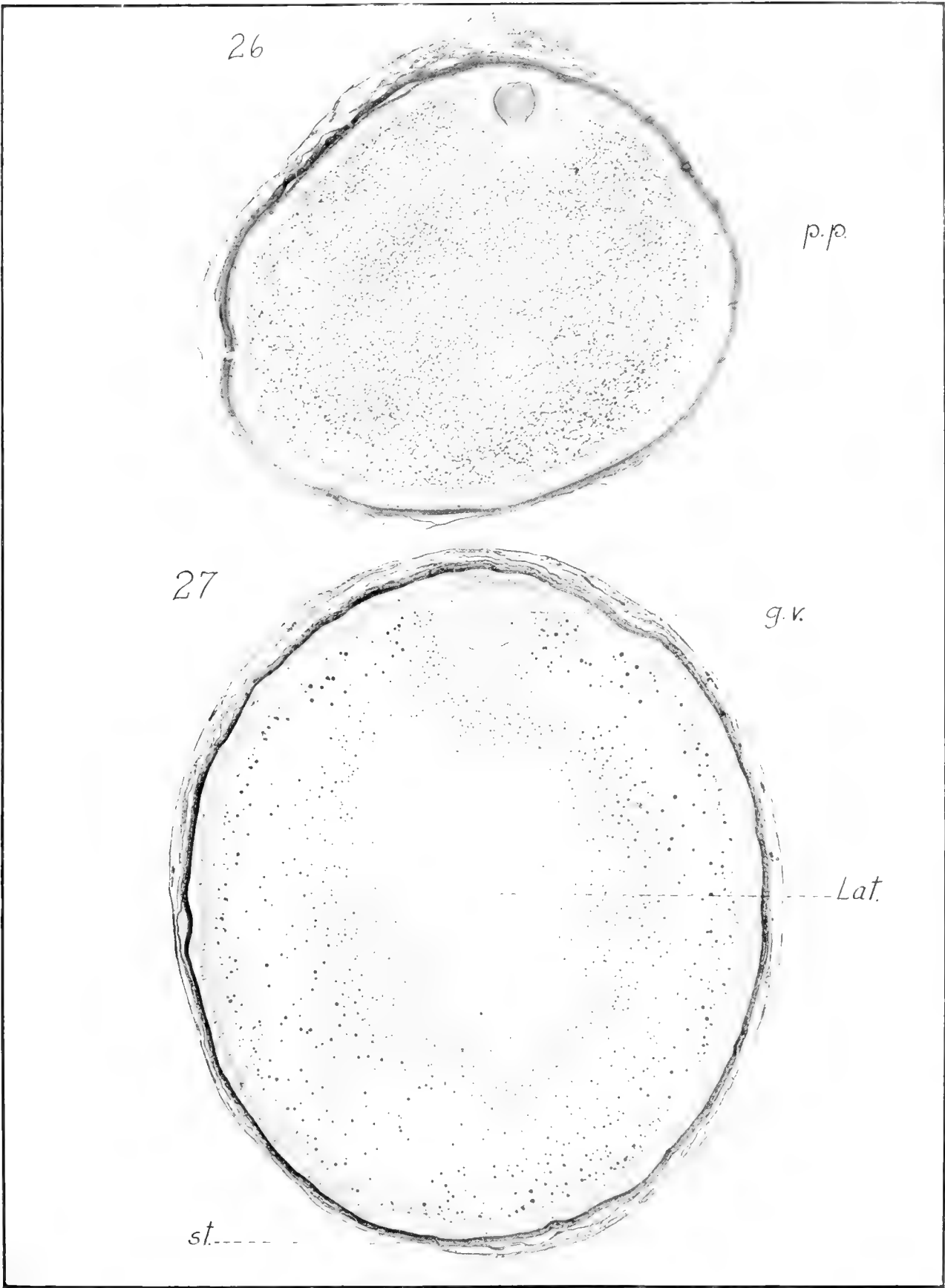


PLATE 6

EXPLANATION OF FIGURES

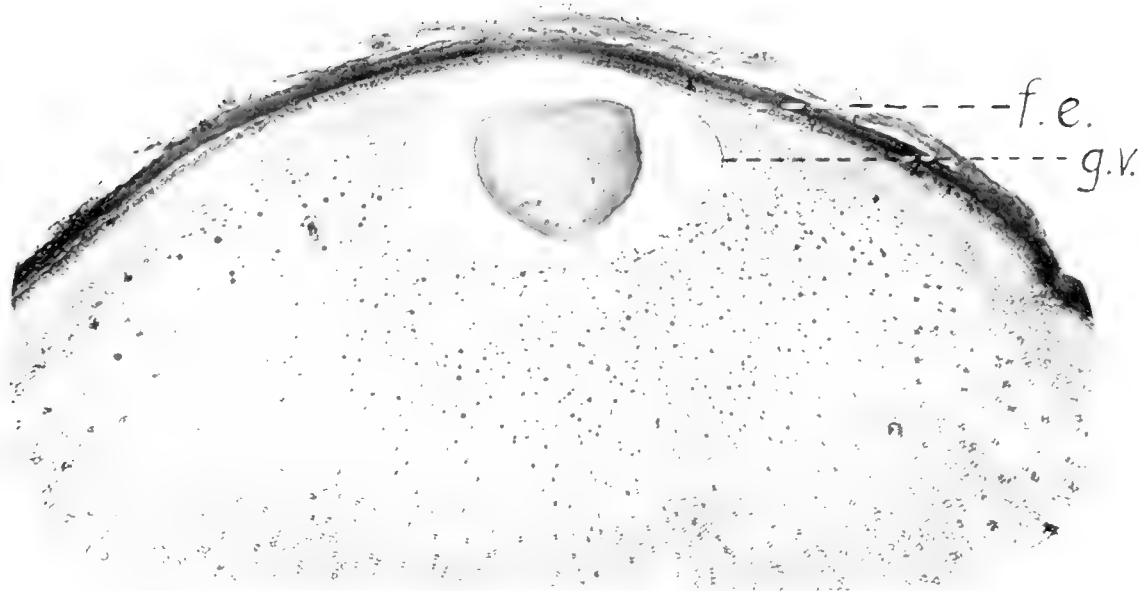
The early development of the blastodisc

28 The animal pole of the 1.74 mm. oocyte shown in fig. 26. Zeiss apochromat 8.0 mm. ocular 2. $\times 100$. The finer reticulum about the germinal vesicle (*g.v.*) is the first trace of the blastodisc which differentiates symmetrically about the germinal vesicle (see fig. 8).

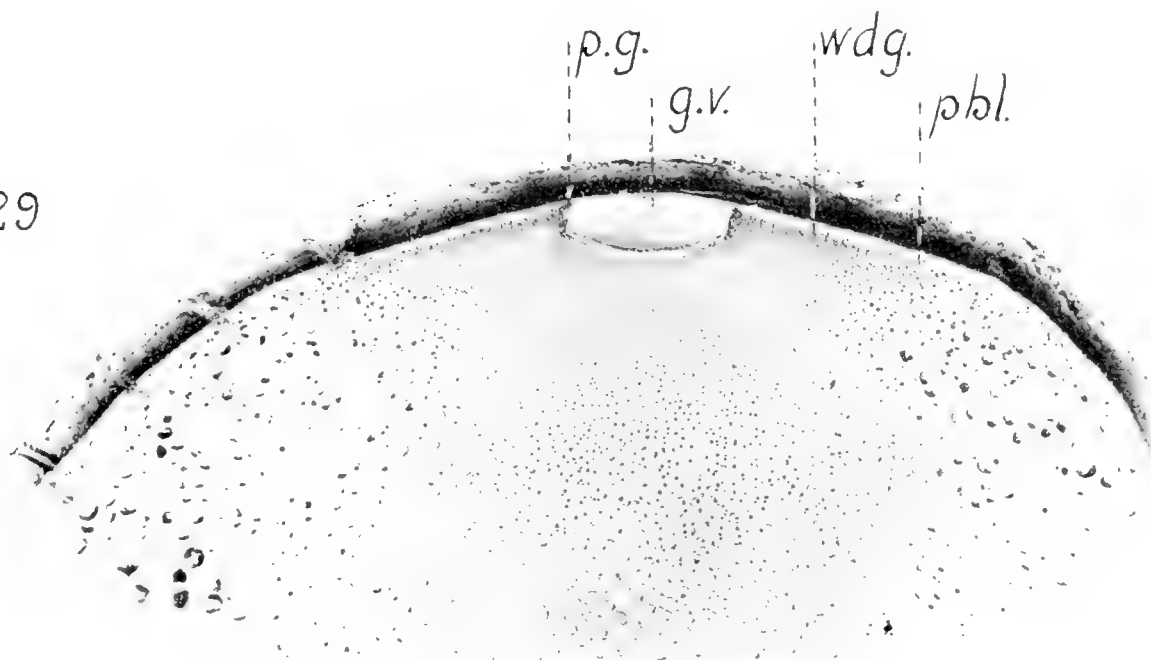
29 An oblique section of the blastodisc of an oocyte of 5.47 mm. (see fig. 7. for surface view). Leitz objective 2. $\times 55$. Marginal (*pbl.*) and central periblast can be distinguished about the finely granular segmental disc. The more deeply staining wedge granules (*wdg.*) have differentiated at the periphery of the disc. The guide line *g.v.* runs to the group of chromosomes and nucleoli at the center of the germinal vesicle. *P.g.*, polar granules. A narrow break on the left separates the wedge granules from the peripheral protoplasm. Final stage of period of differentiation.

30 The blastodisc of an oocyte 8 mm. long axis in life. Beginning of the final growth period. Magnification as in fig. 29. The wedge (*wdg.*) extends from the periblast to the germinal vesicle and the group of polar granules at the edge of the latter is well marked at *p.g.* Note the abrupt transition of the periblast into the surrounding bed of white yolk; there is only a trace of a marginal periblast in this egg.

28



29



30

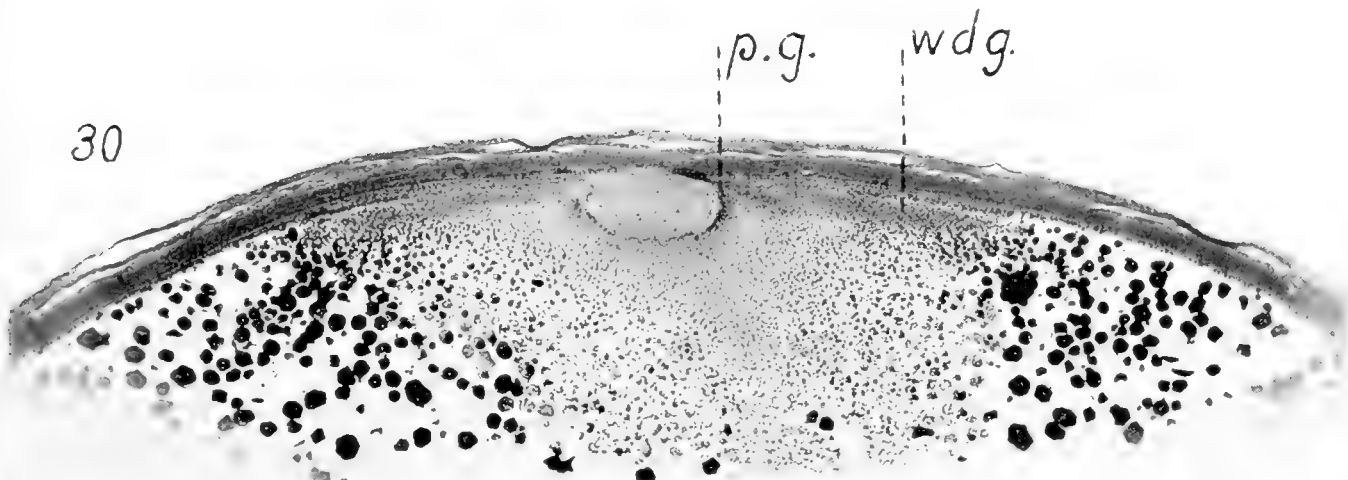


PLATE 7

EXPLANATION OF FIGURES

31 to 34 are from entire follicles photographed in creasote.

31 Follicle 1.1 mm. in long axis. $\times 15$. The focus was changed during the exposure so as to show both the upper surface and the median optical section. The attached pole is up, the long axis extends right and left. The median arteries (*m.a.*) appear on either side of the long axis (the upper one only is in focus.)

32 View of the stigmal pole of an oocyte 2.3 mm. in long axis, showing the two median arteries ending at the stigma, the boundaries of which do not appear clearly. $\times 15$.

33 Side view of a follicle, 5.1 x 4.7 x 4.8 mm. $\times 8$. Focus on upper side and on median plane, to show the bilateral arrangement of the median artery (*m.a.*) with reference to the long axis.

34 Oblique stigmal pole view of the 5.47 mm. oocyte shown in fig. 7. There are no capillaries in the region of the stigma (*st.*)

35 Surface view of the blastodisc of an ovum taken from the middle third of the oviduct at 11 p.m. and photographed by reflected light in the fixative (sublimate-acetic-formol. $\times 6.6$. Fragmentation stage of the segmental disc (*s.d.*), i.e., an early stage in the copulation of the pronuclei. The disc and periblastic zones are clearly elliptical. *pbl.r.*, periblastic ring; *o.p.*, posterior margin of outer periblast.

36 Oblique surface view of the segmental disc of an egg taken from the middle third of the oviduct at 11 p.m. and photographed by reflected light in the same fixative as fig. 35, with one tube of a Zeiss binocular, obj. a₆, oc. 2. $\times 15$. The egg was in a slightly later stage than that shown in fig. 35 and the higher magnification brings out the fragmentation clearly.

37 Two follicles, a day before ovulation, in situ. A trifle larger than natural size. The long axes of the follicles are oriented in the antero-posterior axis of the bird. The median longitudinal section of the larger follicle is shown in fig. 37; *c.l.*, cloaca; *ut.*, 'uterus'; *inf.*, infundibulum.

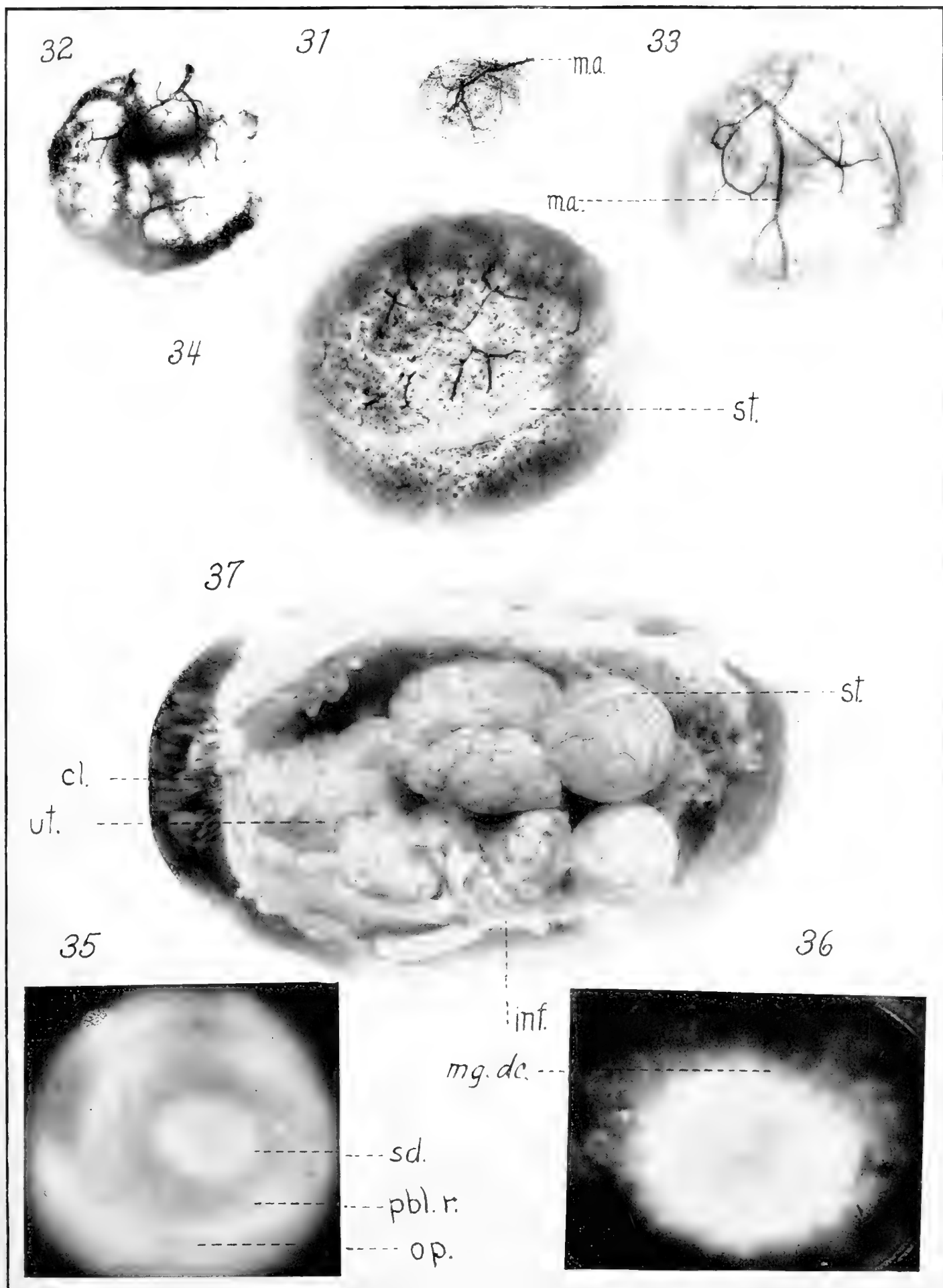


PLATE 8

EXPLANATION OF FIGURES

38 The median surface of an ovarian egg twenty-four hours before ovulation, cut in half through the long and polar axes, and photographed by reflected light. $\times 3$. The central latebra (*lat.*) is distinctly farther from the right hand end of the long axis, the end, namely, which was directed posteriorly ('cloacally') when the bird was opened. The neck of the latebra extends up towards the blastodisc at the periphery. Compare with diagram 2 *E* (in which the concentric layers of yolk are not represented).

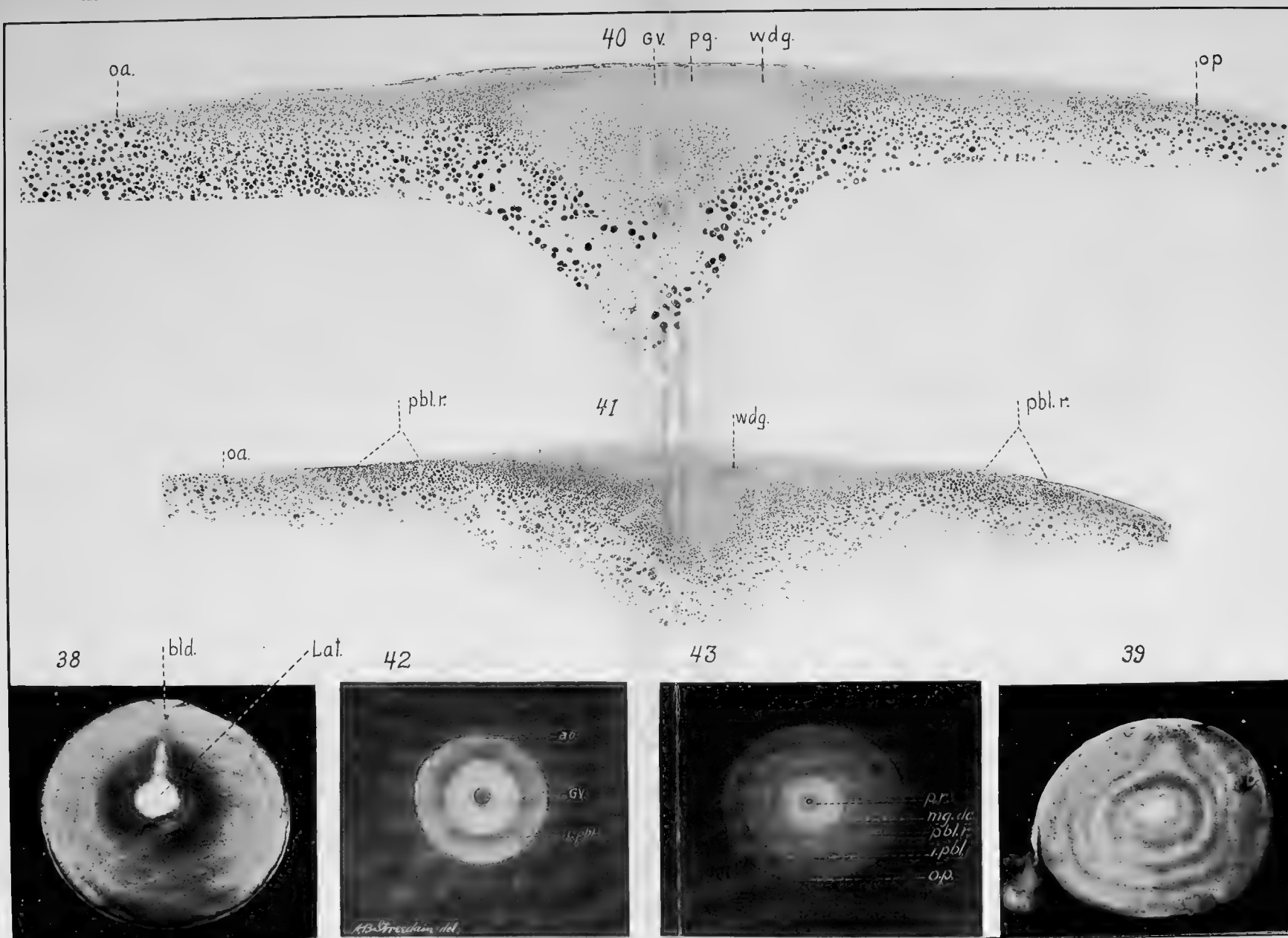
39 A photograph similar to the one shown in fig. 38, from an oviducal egg taken twenty-six hours after fertilization, showing the common type of latebra of oviducal eggs. The section did not cut the blastodisc exactly in the center and so the infundibular extension of the latebra does not appear, but it is indicated by the arrangement of the surrounding layers of yolk. When this is considered and allowance is made for the cracking of the yolk at the infundibular end, the center of the latebra is 2 mm. nearer this end of the long axis. The hole in the yolk at the right was made by the pin inserted to mark the cloacal end of the egg when it was obtained.

40 A photograph of a parasagittal section of the blastodisc of an oocyte, 17.8 x 16.9 x 16.9 mm., taken about 26 hours before ovulation, fixed in sublimate-acetic and stained with neutral gentian. Leitz obj. 3, $\times 56$. The 'wedge' granules (*wdg.*) form a collar around and tapering toward the germinal vesicle (*g.v.*), which is immediately surrounded by the polar granules, (*p.g.*). *o.a.* and *o.p.* mark the anterior and posterior margins of the outer periblastic zone of the blastodisc.

41 A photograph of a parasagittal section of an egg with the first cleavage spindle formed, taken from the entrance to the shell gland at 11 p.m., fixed in sublimate-acetic and stained with neutral gentian. Leitz obj. 3, $\times 48$. The wedge (*wdg.*) is clearly defined posteriorly and absent anteriorly. The periblastic ring, (*pbl.r.*) is of the diffuse type.

42 Surface view of the blastodisc of an oocyte removed from its follicle about forty-five hours before ovulation would have occurred normally. A copy of a drawing made from the living ovum. $\times 10$. At the center is the germinal vesicle (*g.v.*), with the narrow opaque (white) zone of polar granules (cf. fig. 40, *p.g.*). The segmental disc is circular and appears light; at its outer margin the transparent (dark) inner periblastic zone (*i.pbl.*) is differentiating. At the outer margin of the periblast are light spots which represent groups of periblastic granules. These have not yet appeared anteriorly at *a.o.* The antero-posterior axis indicated by this difference in the structure of the periblast formed an angle of 65 to 70 degrees to the long axis of the oocyte.

43 The surface view of the blastodisc of a fertilized egg taken from the infundibular third of the oviduct and drawn after one and one quarter hours of incubation. Magnified ten times. A copy of one of a series of drawings of the living egg showing the formation of the periblastic ring (*pbl.r.*) (cf. fig. 35).



I. A FURTHER STUDY OF THE CHROMOSOMES OF THE REDUVIIDAE. II. THE NUCLEOLUS IN THE YOUNG OOCYTES AND ORIGIN OF THE OVA IN GELASTOCORIS

FERNANDUS PAYNE

From the Zoological Laboratory of Indiana University¹

TEN FIGURES

I. A FURTHER STUDY OF THE CHROMOSOMES OF THE REDUVIIDAE

Since describing several irregularities of chromosome distribution in this family ('09 and '10), I have been seeking further information in regard to the origin of such irregularities. I expressed the view ('09) that these irregularities probably arose by the large idiochromosome breaking up into two, three, four, or five elements. While I had no authority for believing it, I looked upon these changes as recent ones, and hoped to find a species in which such a change was taking place. During the past summer, I made a collecting trip through the West and South. A study of this material has thrown some doubt on this view. I have five species of *Sinea* (*diadema*, *rileyi*, *confusa*, *complexa*, *spinipes*) and they are distributed from Massachusetts to California. Four of them (*spinipes*, *confusa*, *complexa*, and *diadema*) show the same number and size relations of the chromosomes and the same type of distribution as *Sinea diadema* (Payne, '09). From this fact it would seem that both the number of chromosomes and the type of distribution existed before the genus split up into these four species and before it became distributed over such a wide area. Otherwise it is rather difficult to explain this constancy in number and behavior of the chromosomes in the four species. On the other hand a study of the chromosomes of *Sinea*

¹ Contribution No. 124.

rileyi, which presents a type of distribution similar to that of *Acholla multispinosa*, might lead us to the opposite conclusion. If there were present in the parent species a type of distribution similar to that of *Sinea diadema* we would have to assume a further splitting up of these chromosomes in order to reach the condition found in *Sinea rileyi*. Such an assumption would only complicate matters. In this case it would seem that this species split off from the parent one before any breaking up of the single large idiochromosome had occurred, assuming, of course, that a single pair of idiochromosomes was the original condition. In all probability then, the breaking up of the large idiochromosome has occurred independently in the parent species and in *Sinea rileyi* and, of course, may have occurred at any time since the origin of the species.

As the type of chromosome distribution found in *Sinea rileyi* has been described in only one other species it seems worth while to describe it somewhat in detail. Unfortunately, I have a small amount of material and it does not show spermatogonial or oogonial divisions. However, first and second spermatocyte divisions are in abundance and, I think, indicate clearly that the type of distribution is similar to that of *Acholla multispinosa* (Payne, '09 and '10). The first spermatocyte division (fig. 1, *A* and *B*) shows eighteen chromosomes, six of which are smaller than the remaining twelve. All divide in this division so that each secondary spermatocyte receives eighteen chromosomes. There is no definite arrangement of the chromosomes in the first division but the characteristic regrouping, found in the remainder of the Reduviidae, occurs in the second division. The twelve larger chromosomes are arranged in an irregular ring with the six smaller ones forming a hexad group in the middle. Five of these six lie in one plane while the other one lies in a different plane either above or below the five. Figure 1, *D* and *E* are pole views of this division showing the twelve chromosomes in the ring and the five which lie in one plane in the middle. Figure 1, *F* is the same as *E*, but drawn at a different focus to show the one chromosome in the middle instead of the five. Figure 1, *G* is a slightly oblique view showing the hexad group. Figure 1, *H* is a

side view and shows clearly the relative positions of the *X* and *Y* elements. Only four of the five chromosomes show in this figure. Although no anaphases are present in my material, I think we may safely conclude, from what has been found in the rest of the family, that the twelve chromosomes in the ring divide equally, while the five members of the hexad group which lie in one plane pass to one pole undivided and the one below or above passes

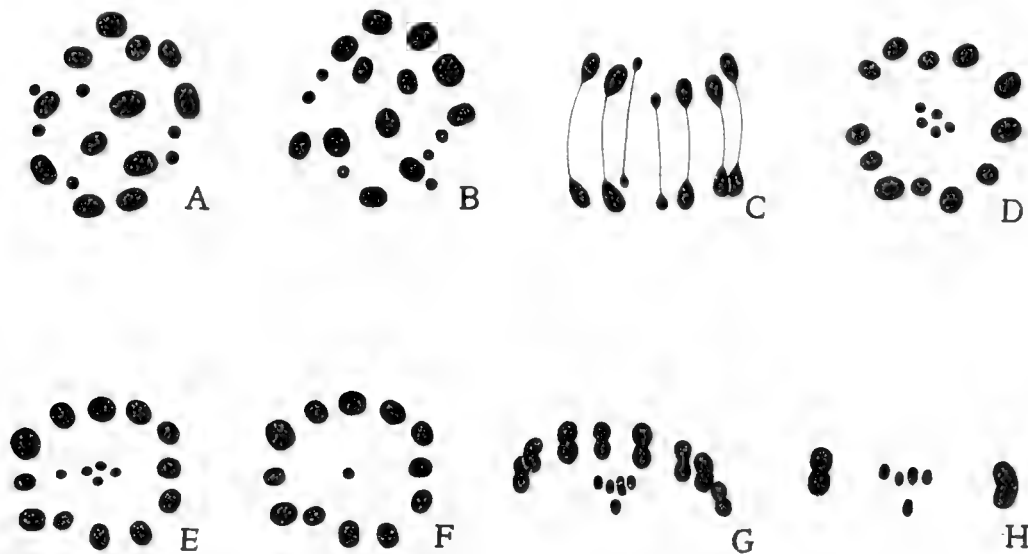


Fig. 1 *Sinea rileyi* Montd. *A* and *B*, metaphase plates of the first spermatocyte division showing eighteen chromosomes, six of which are noticeably smaller than the remaining twelve; *C*, a side view of an anaphase of the first spermatocyte division showing that the small chromosomes divide in this division; *D* and *E*, metaphase plates of the second spermatocyte division with twelve chromosomes in the ring and the five chromosomes (*X* element of Wilson) which lie in one plane in the middle; *F* is the same as *E* except it is drawn at a different focus to show the single chromosome (*Y* element); *G* is a slightly oblique view of the second division to show the hexad group in the middle; *H* is a side view of the second division and shows the relative positions of the *X* and *Y* elements (only four of the five chromosomes show in this figure). $\times 2275$.

to the opposite pole undivided. This would give two classes of spermatozoa, one with thirteen and one with seventeen chromosomes and the fertilization formula would be as follows:

SPERM		EGG		
13	+	17	=	30♂
17	+	17	=	34♀

The most striking difference between the chromosomes of *Acholla* and *Sinea* is in the size relations of the idiochromosomes. In *Sinea* all six of them are practically the same size while in *Acholla* three of them are very small, two intermediate and one (the *Y* element) very large.

A study of the chromosomes of *Pnirontis modesta* Banks has revealed a type of distribution new to the family and similar to that described for *Gelastocoris oculatus* (Payne, '09). I have only one specimen, but I believe it shows sufficient stages to justify the above conclusion. Figure 2, *A*, *B*, and *C* show the spermatogonial group with twenty-five chromosomes, three of which are very small. In the first spermatocyte division (fig. 2, *D* and *E*), there are fifteen chromosomes. Again three of these are very small. Of the remaining twelve, ten are larger and two intermediate between the large and small ones. There is no definite arrangement of the chromosomes and all divide in this division so that all the secondary spermatocytes receive fifteen chromosomes. In the second division there is the characteristic regrouping found in all the Reduviidae. The ten large chromosomes form a more or less irregular ring with the two intermediate and three small ones forming a pentad group in the middle. This group was somewhat difficult to analyze on account of the small size of the chromosomes and the fact that they lie so close together. The most favorable metaphases of the second division (fig. 2, *F* and *G*) show, however, that four of these five, the three small and one intermediate lie in one plane while the other intermediate one lies below or above them. Figure 2, *H*, the best side view obtainable of the second division, shows this one chromosome and its relative position with respect to the other four, which in this figure are massed together as one. Although no anaphases are present showing the method of this division, I think there can be little doubt, judging by the number relations in the spermatogonial and first spermatocyte divisions and also from the analogy here to the second spermatocyte divisions in the other Reduviidae, that the ten chromosomes in the ring divide equally, while the four members of the pentad group (*X* element), which lie in one plane, pass to one pole undivided and the other one passes un-

divided to the opposite pole. This gives two classes of spermatozoa, one with fourteen chromosomes and the other with eleven, and the fertilization formula would be as follows:

SPERM		EGG		
11	+	14	=	25♂
14	+	14	=	28♀

The chromosomes of *Pselliodes cinctus* Fabr. also deserve mention. As shown in figure 3, the type of distribution is similar to

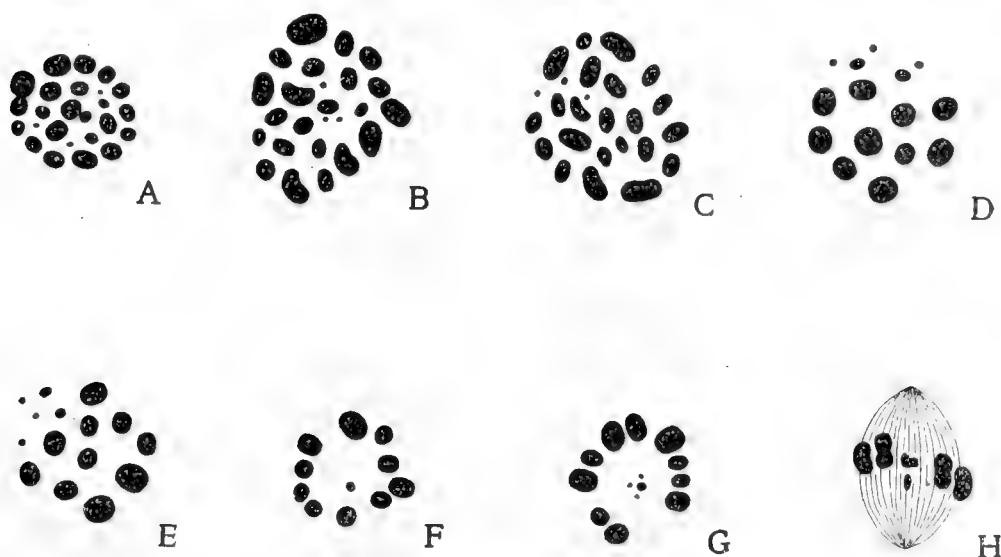


Fig. 2 *Pnirontis modesta* Banks. *A*, *B* and *C*, spermatogonial divisions showing twenty-five chromosomes; *D* and *E*, first spermatocyte divisions with fifteen chromosomes (the five small ones are the idiochromosomes); *F*, second spermatocyte division, showing ten chromosomes in the ring and the one idiochromosome (*Y* element) which lies in a different plane from the other four; *G*, second spermatocyte division with ten chromosomes in the ring and the four idiochromosomes which lie in the same plane; *H*, side view of the second maturation division showing the single idiochromosome below and the four above massed together as a single body. $\times 2275$.

that described for *Prionidus cristatus* and *Sinea diadema* (Payne, '09). The size relations of the idiochromosomes, however, are different. In *Sinea* all four were practically the same size; in *Prionidus* one was slightly larger than the other three; while in *Pselliodes* the single chromosome, the homologue of the small idiochromosome (*Y* element), is much larger than the others;

so much larger, in fact, that it undoubtedly contains a larger quantity of chromatin than the other three combined. In this respect *Pselliodes* resembles *Acholla multispinosa* (Payne, '09 and '10).

The paper of Della Valle ('09) again brought to the surface the question of the variation of the number of chromosomes within a given species and individual. This paper has been ably dis-

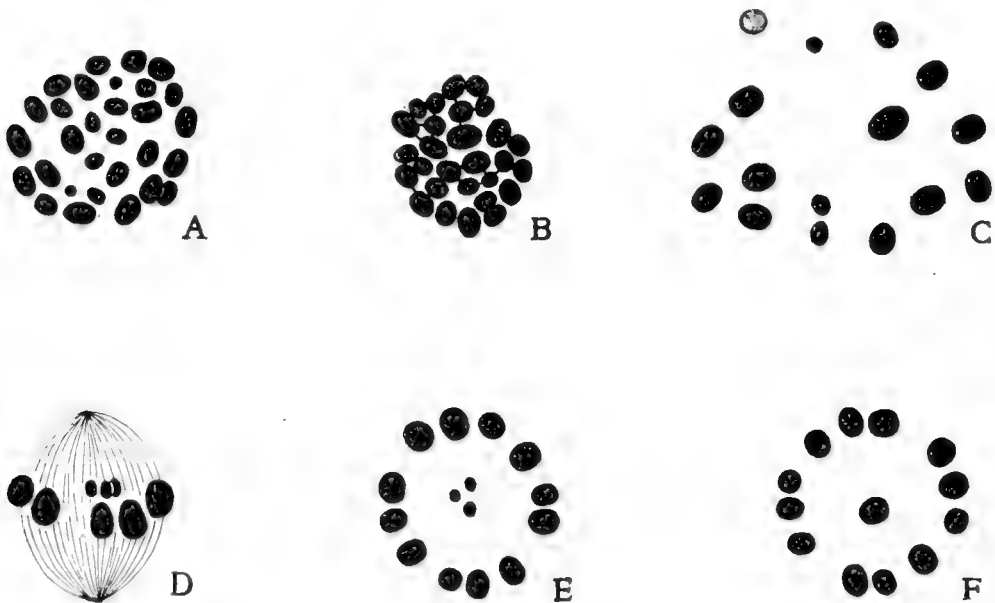


Fig. 3 *Pselliodes cinctus* Fabr. *A*, oogonial division with thirty chromosomes; *B*, spermatogonial division with twenty-eight chromosomes; *C*, first spermatocyte division with sixteen chromosomes; *D*, side view of the second spermatocyte division, showing the four idiochromosomes as a tetrad group in the middle and the relative size of the four; *E* and *F*, metaphase plates of the second spermatocyte division, *E* showing the three small idiochromosomes in one plane in the middle, and *F* showing only the single large idiochromosome in the middle. $\times 2275$.

cussed by Montgomery ('10) and Wilson ('10). These authors, while they admit Della Valle has done a good service in collecting a large amount of data against the prevalent notion of chromosomal constancy, believe he has been unjust in his criticisms and that his evidence is insufficient for his sweeping conclusions. It is not my intention to discuss any of these papers, but to describe a case of apparent variation and give what I think to be its explanation.

Apiomeris crassipes is one of the Reduviidae in which we find a single slightly unequal pair of idiochromosomes. The spermatogonial number is twenty-four, the first spermatocyte thirteen and the second spermatocyte twelve. I made twelve counts of the first division and thirty-four of the second. These counts were made from metaphase plates which were perfectly flat and in which the chromosomes were well separated. The counts of the first division showed a range from thirteen to sixteen; one with thirteen, six with fourteen, four with fifteen, and one with sixteen. Figure 4, *A, B, C, D, E* and *F* are metaphase plates showing these variations and I think that even the most optimistic will admit that these figures seem to show a real variation. Things look somewhat differently though when such figures are viewed from the side (fig. 4, *G, H* and *I*). These figures show clearly that some of the bodies, which in pole view appear to be chromosomes, are not really chromosomes at all but in all probability are yolk granules. At any rate, they do not behave as chromosomes and, it seems to me, behavior is about the best test for a chromosome. To be sure, chromosomes differ in their behavior and within the last few years several types of behavior have been described, but these bodies do not behave like any of the described types. The chromosomes are clearly bipartite. The granules are spherical and never at any stage show signs of constriction. They may or may not lie in the same plane as the chromosomes.

Counts of the second maturation division also show similar variations. The normal number in this division is twelve. Out of thirty-four counts, there were six with twelve, eleven with thirteen, six with fourteen and eleven with fifteen bodies resembling chromosomes. Some of these variations are shown in figure 4, *J* and *L*. Side views (fig. 4, *M* and *N*) of these metaphases again show the granules as spherical unconstricted bodies.

I have no anaphases of *Apiomeris* showing conclusively that these granules do not divide, but have made some counts of the second maturation division in *Conorhinus* where anaphases are present. The normal number of chromosomes in this division of *Conorhinus* is thirteen, but since one of the triad group in the middle lies below the two, only twelve show. One hundred

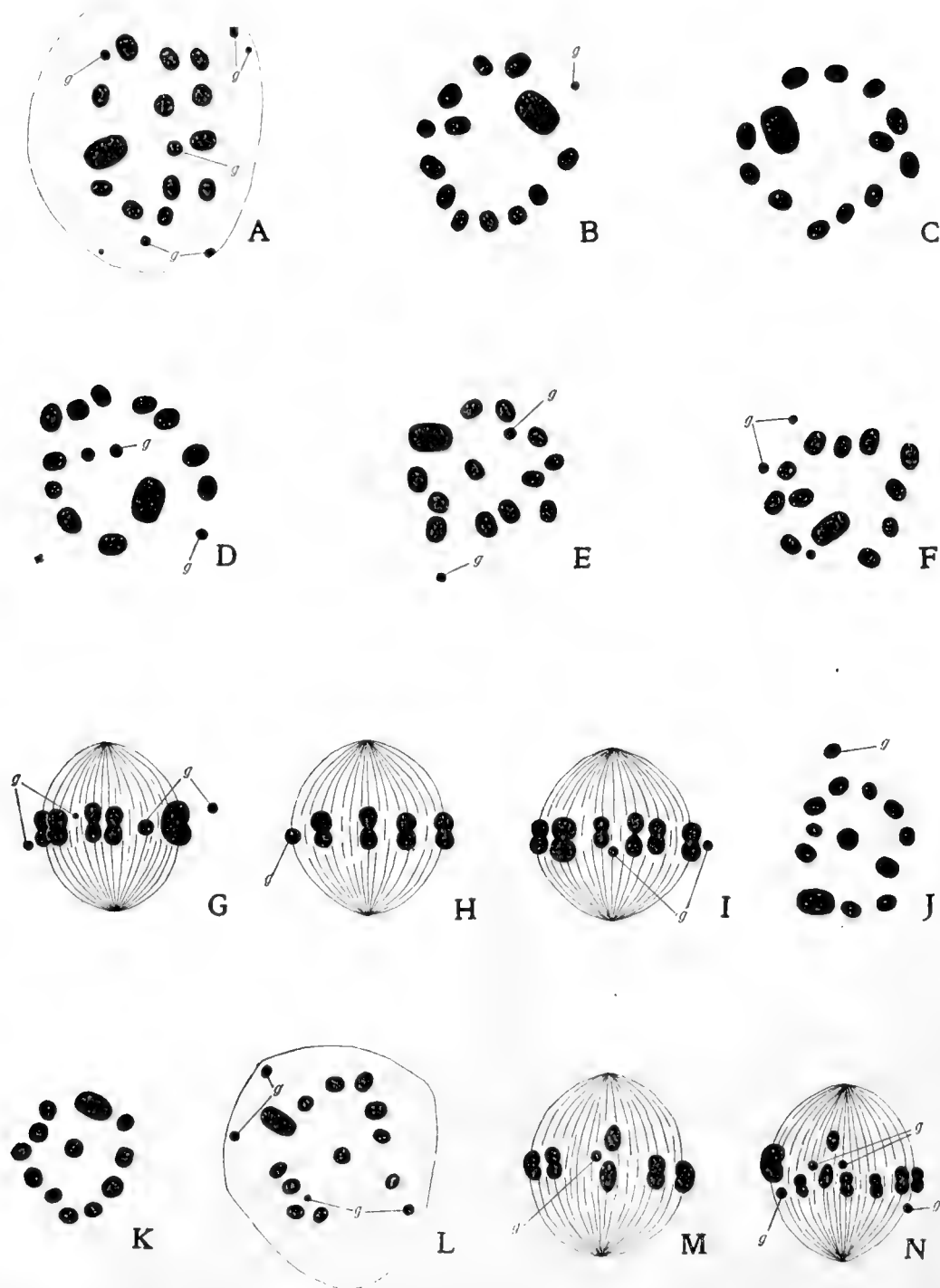


Fig. 4 *Apiomerus crassipes*. A, B, D, E and F, pole views of the first spermatocyte division showing an apparent variation in the number of chromosomes (the bodies marked 'g' are probably yolk granules); C, first spermatocyte division showing the normal number; G, H and I, side views of the first spermatocyte division showing the chromosomes constricted and the spherical yolk granules; J, K and L, pole views of the second spermatocyte division showing an apparent variation in number (K shows the normal number); M and N, side views of the second spermatocyte division, showing the granules among the chromosomes. $\times 2275$.

and eighty-five counts gave one hundred and fifty cases with the normal number and thirty-five with thirteen and fourteen. Figure 5, *A*, *B*, *C* and *D* show polar views of such metaphases. In *C* and *D* the granules are near the periphery of the cell and perhaps in these cases would not be taken for chromosomes, *E*, *F*, *G* and *H* show clearly that these granules may lie in any plane and that they do not divide.

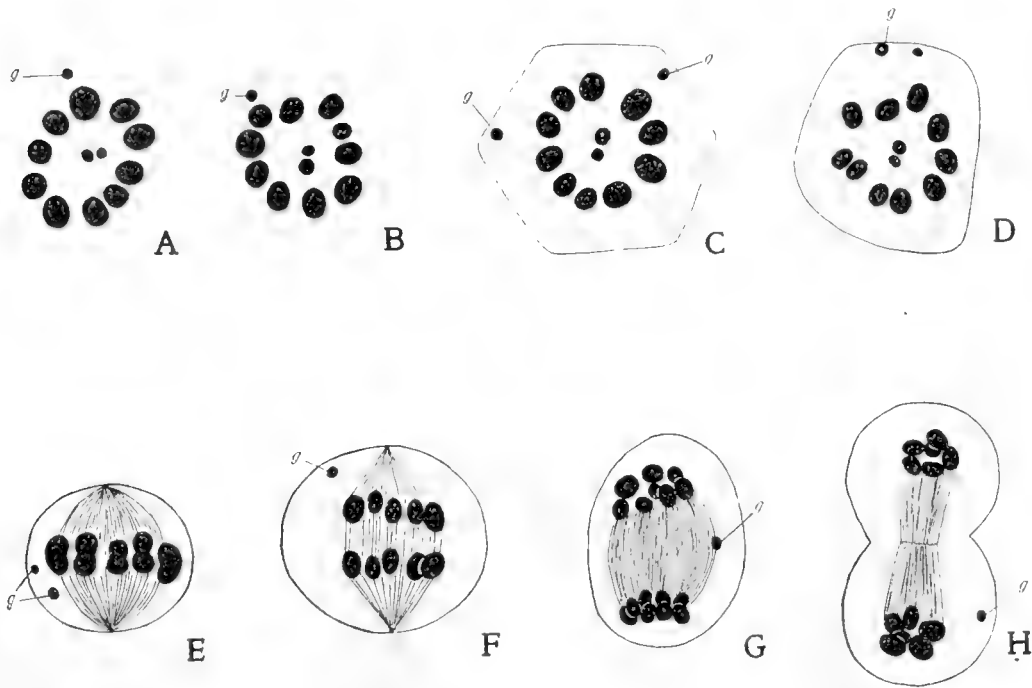


Fig. 5 *Conorhinus sanguisugus* Lec. *A* and *B*, metaphase plates of the second spermatocyte division showing a single granule (*g*) lying just outside the ring of chromosomes; *C* and *D*, metaphase plates of the second maturation division showing two granules near the periphery of the cells; *E*, metaphase plate, side view showing two small granules; *F*, *G* and *H*, anaphases, side view, of the second maturation division, showing that the granules do not divide and may pass into either daughter cell. $\times 2275$.

In these cases, then, what might be termed chromosomal variation is not variation at all, but is due to the presence of yolk granules which may happen to lie in the metaphase plate. It is a significant fact that in these counts not a single one fell below the normal number. This is also illustrated in *Reduvius personatus* where ninety-eight counts of the first maturation division gave ninety-six with the normal number (twelve) and two with thirteen. Eighty-four counts of the second division gave

seventy-seven with the normal number (eleven) and seven with twelve. If this be a real chromosomal variation why should the variations always be greater than the normal number and never less?

I have included two figures of *Gelastocoris* (fig. 6, *A* and *B*) where a large number of undoubted yolk granules are present. *A* is a prophase of the first maturation division before the nuclear wall breaks down and *B* a metaphase plate showing how difficult it is, looking at a single cell, to differentiate between granules and chromosomes.

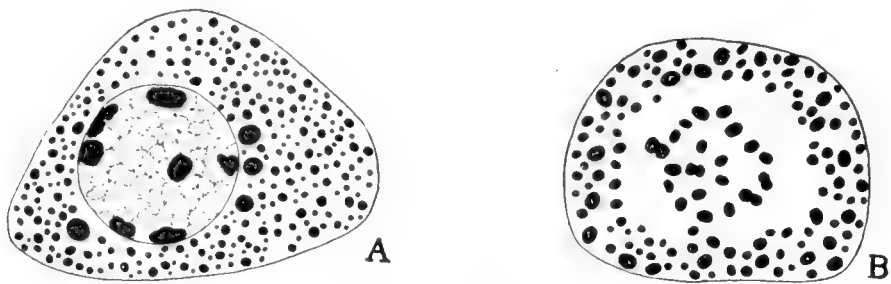


Fig. 6 *Gelastocoris oculatus* Fabr. *A*, prophase of the first spermatocyte division showing many yolk granules outside the nucleus and their close resemblance to chromosomes; *B*, metaphase plate, first spermatocyte division showing the chromosomes in the middle and the granules near the periphery of the cell and also the close similarity between the two. $\times 860$.

II. THE NUCLEOLUS IN THE YOUNG OOCYTES AND ORIGIN OF THE OVA IN GELASTOCORIS

In a recent paper ('11) Foot and Strobell describe a chromosome nucleolus in the oogonial cells of *Protenor* and also describe the origin of the two large idiochromosomes from it at the time of mitotic cell division. At the time this paper appeared, I was working on the nucleolus in the ovaries of *Gelastocoris oculatus*. While the two forms have some things in common there are several points of divergence. As stated, the nucleolus in *Protenor* is confined to the oogonial cells. This is not the case in *Gelastocoris*. Here it appears after the last oogonial division, in the early oocyte, and persists until shortly after synapsis. Figure 7, *A* is an oogonial cell in which there is no indication of a nucleolus. Figure 7, *B* shows an early oocyte and the beginning of the formation of the nucleo-

lus. In this early stage it seems to be pretty conclusive that the nucleolus is formed by an aggregation of chromatin. This agrees with Foot and Strobell's interpretation of the formation of the nucleolus in *Protenor*. Figure 7, *C*, *D* and *E* are later stages in the development of this nucleolus. These figures show that it increases in size rather rapidly and becomes large in proportion to the size of the nucleus. This rapid growth along with the fact

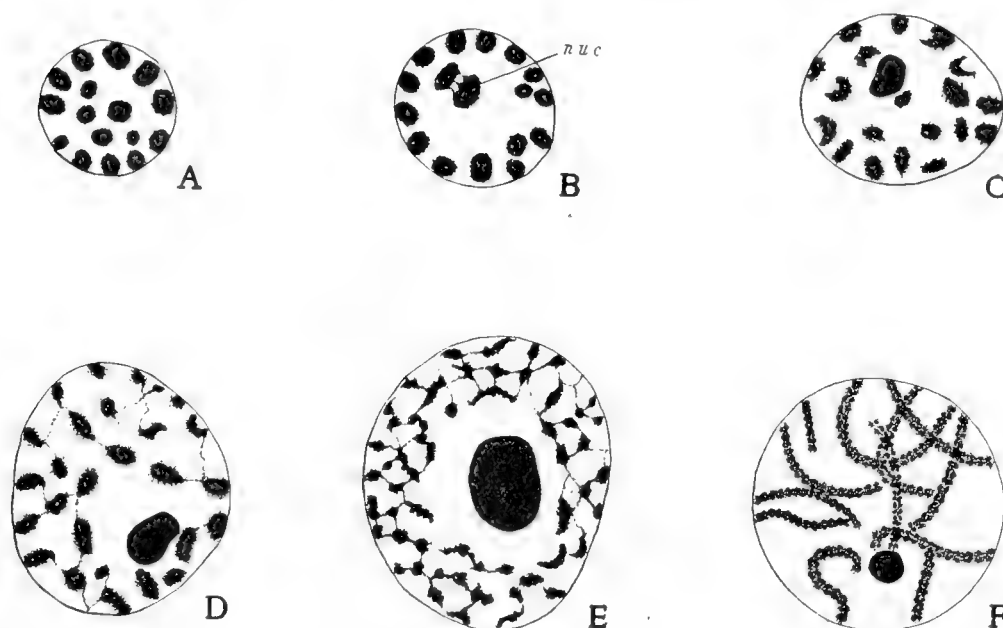


Fig. 7 *Gelastocoris oculatus* Fabr. *A*, an oogonial nucleus showing no nucleolus is present; *B*, a young oocyte nucleus in which some of the chromatin is collecting together to form the nucleolus (*nuc.*); *C* and *D*, young oocyte nuclei, showing the growth of the nucleolus; *E*, young oocyte nucleus showing the nucleolus at approximately its maximum size (in this case the nucleolus is not stained so intensely as in the others and eight darker bodies lie imbedded in it); *F*, an oocyte nucleus shortly after synapsis with the chromatin as doubly split threads and the nucleolus much reduced in size. $\times 2275$.

that no chromatin is added after its beginning indicate that its rapid growth is due to the addition of something other than chromatin. Figure 7, *E* also indicates the same thing since here the nucleolus does not stain uniformly but darker bodies can be seen within it. In this case there are eight of these darker bodies and, while the evidence is not conclusive, it seems probable that they are the idiochromosomes and the whole structure is a nucleolus or plasmosome in which the idiochromosomes are imbedded.

So striking is the resemblance to the condition described in the growth period of the spermatocytes of *Pironidus* (Payne, '09) where beyond a doubt, the idiochromosomes are imbedded in a plasmosome, that this conclusion seems very probable. At the time of synapsis or shortly after (fig. 7 *F*), the nucleolus is much reduced in size and later it completely disappears (fig. 8 *B, C, D, E* and *F*, serial sections of a single young oocyte). Foot and Strobell believe, on account of size relations, that the nucleolus in *Protenor* represents something more than the two large idiochromosomes. Is it not possible that here also, the nucleolus is one in which the chromosomes are imbedded and that it disappears at the time of cell division, leaving only the chromosomes? The main difference then between the nucleolus in the ovaries of *Protenor* and *Gelastocoris* is the period during which it persists.

In this same paper Foot and Strobell describe the terminal chambers of the ovaries of *Protenor* as differentiated into three distinct zones. They designate these zones as *A, B* and *C*, *A* being the apex of the terminal chamber. *B* is the middle zone and is characterized by large nuclei which vary in form and structure and which stain intensely with chromatin stains. Zones *A* and *C* are somewhat alike, the nuclei being smaller than in zone *B* and staining less intensely. These same three zones have been described by other workers (Will, '85; Korschelt, '86, and Preusse, '95). So far all these workers agree. They further agree that the larger nuclei of zone *B* arise by a process of growth from the nuclei of zone *A*. The difference of opinion arises as to the interpretation of the nuclei of zone *B* and the origin of the nuclei of zone *C* and hence the origin of the ova. Korschelt holds that the nuclei of zone *B* are purely nourishing nuclei which disintegrate to form food for the developing ova, and that the nuclei of zone *C* arise by a continuation of the nuclei unchanged from zone *A*. On the other hand Foot and Strobell ('11) with Will ('85) claim that the nuclei of zone *B* are not all nourishing nuclei and that the nuclei of zone *C* arise in the main by fragmentation or amitotic division from the large nuclei of zone *B*.

While the end chambers in the ovary of *Gelastocoris* are not divided into three distinct zones, the material is very favorable

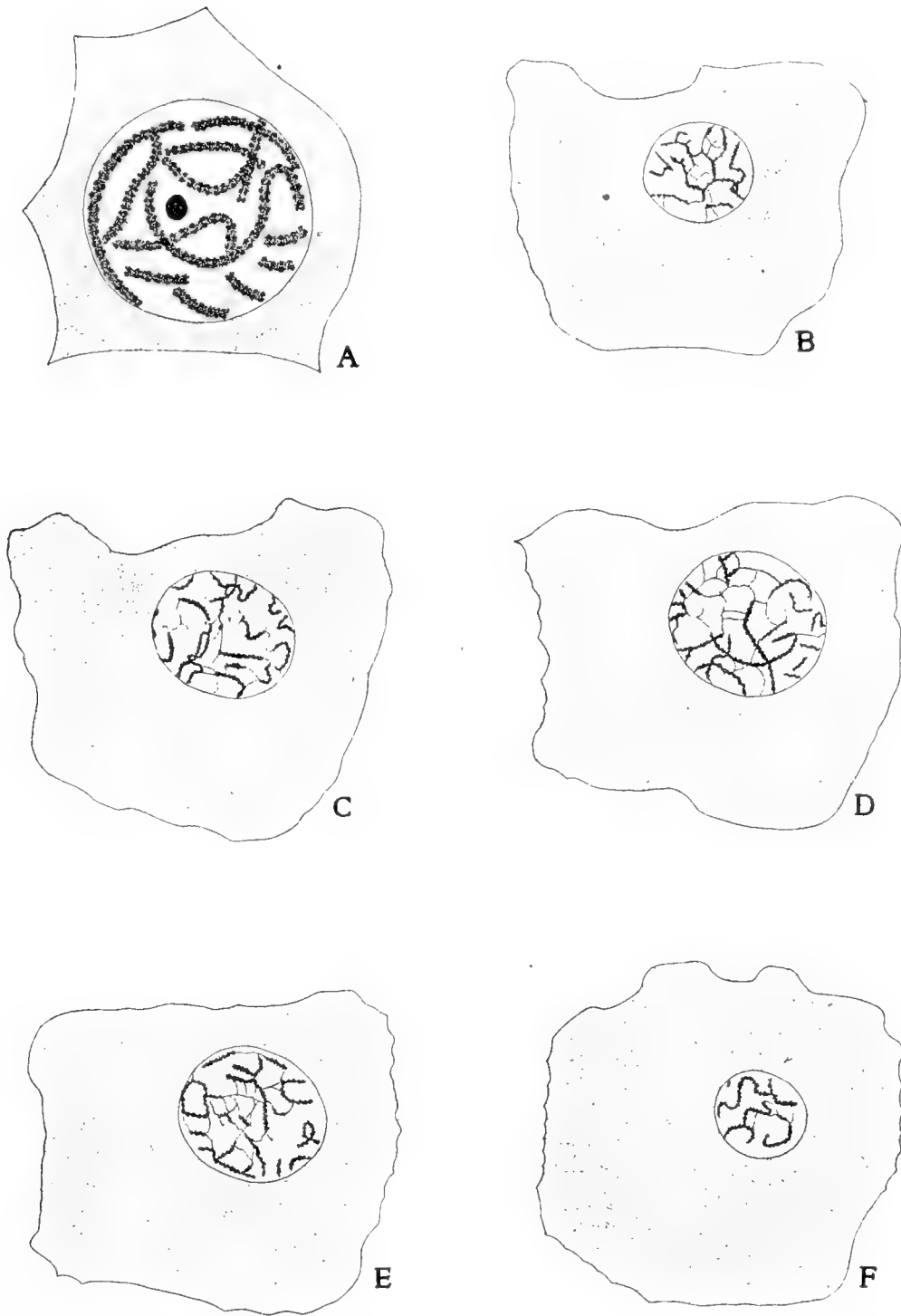


Fig. 8 *Gelastocoris oculatus* Fabr. A, young oocyte just as it starts down the tube and as the cytoplasm is beginning to collect about the nucleus (the chromatin and nucleolus in this nucleus are in practically the same condition as in fig. 7, F); B, C, D, E and F, serial sections of the same oocyte (a little older than A) showing no nucleolus is present at this time. A, $\times 2275$; B, C, D, E and F, $\times 860$.

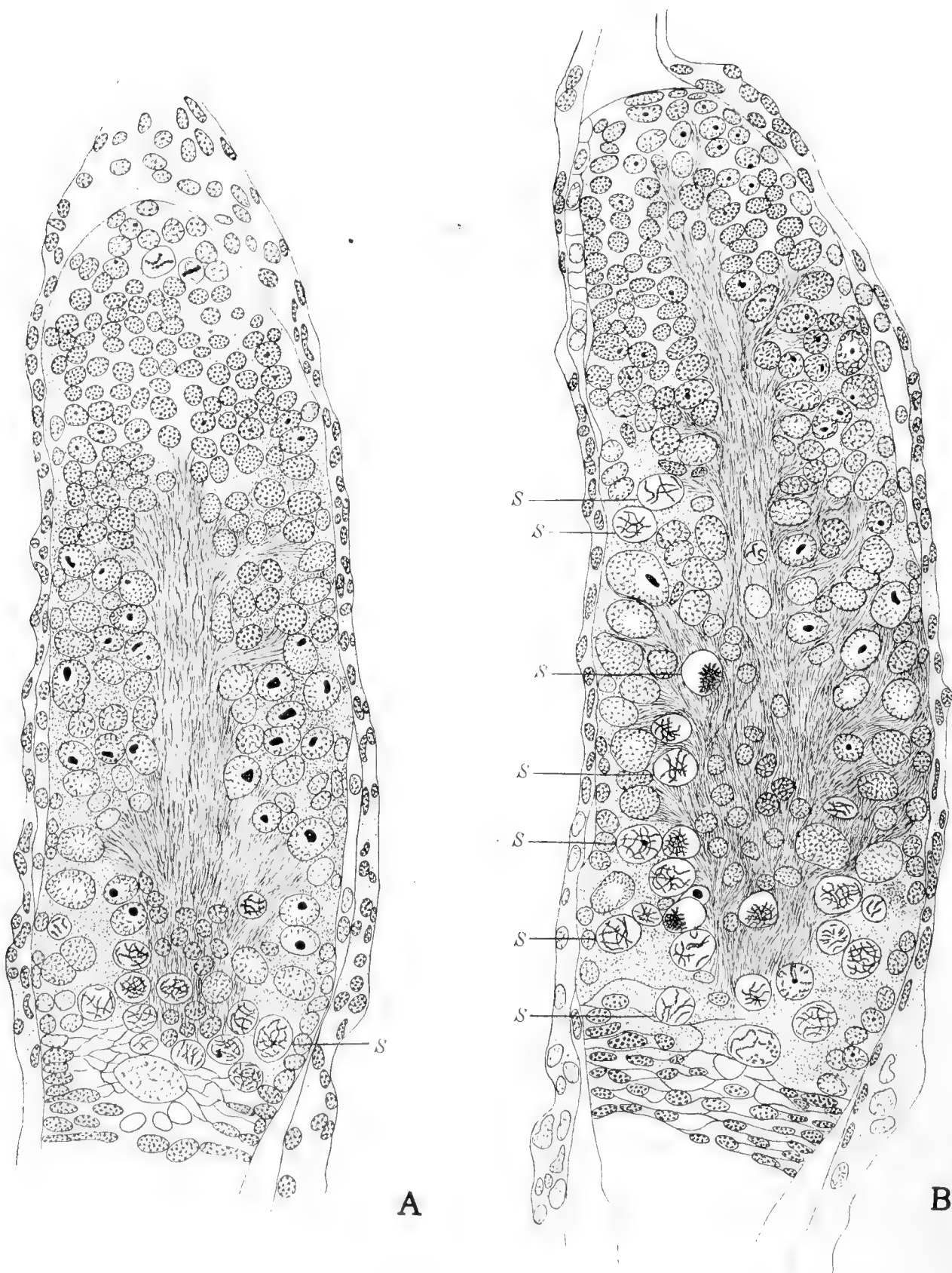


Fig. 9 *Gelastocoris oculatus* Fabr. *A* and *B*, longitudinal sections through two terminal chambers of two tubes from the same ovary. These sections are taken from a young ovary and illustrate the gradual transition of the small nuclei of the apex into the large food nuclei and into the oocytes. They also show that the young oocytes (*S*) are scattered among the food nuclei and that there are not three distinct zones of nuclei as in *Protenor*. $\times 467$.

for the study of the continuity of the ova from the tip of the end chamber to maturity. Figure 9, *A* and *B* are two drawings of the end chambers from a young ovary. It will be seen that both show two zones distinctly, the apex of small nuclei corresponding to zone *A* and the rest of the end chamber composed principally of large nuclei corresponding to zone *B* of Protenor. There is no distinct region, however, which can be called zone *C*. A few small nuclei (fig. 9, *A* and *B*) are found near the lower end of the region which corresponds to zone *B* and it is possible that these nuclei are the representatives of zone *C*, although they are not confined to a definite region as in Protenor. As to the origin of these small nuclei in *Gelastocoris*, I am not quite certain. I am willing to grant that they may arise by migration, unchanged, from the apex or that they may arise by fragmentation of the large nuclei. In this case it is of little concern where they arise, as the evidence in *Gelastocoris* proves conclusively, I think, that the ova do not arise from these small nuclei. Figures 9, *A* and *B*, and 10 show a number of nuclei in the synaptic stage (*S*). That these nuclei in this stage are the true oocytes is demonstrated in figures 7, *F* and 8, *A*. Figure 8, *A* shows a young oocyte at the base of the terminal chamber and as it starts down the tube (similar in position to the oocyte *ov* in fig. 10). The cytoplasm is just beginning to form around the nucleus and the chromatin is in practically the same condition as in figure 7, *F*, where the double threads are coming out of the contraction phase. This clearly demonstrates that the oocyte starts down the tube shortly after the synaptic stage. Hence my conclusion that all the nuclei in the synaptic stage are young oocytes. As is shown in figure 9, *B*, these nuclei in the synaptic stage (*S*) are scattered among the large nourishing nuclei, extending up as far as the border line between the large nuclei and the small nuclei of the apex. This figure along with figure 7 shows that these nuclei undoubtedly arise by the growth of the small oogonial nuclei at the apex and hence in this form there is no break in the continuity of the cells from the oogonial stage to the fully developed ova.

While the evidence in *Gelastocoris* warrants the above conclusion, I by no means wish to generalize and say that this must

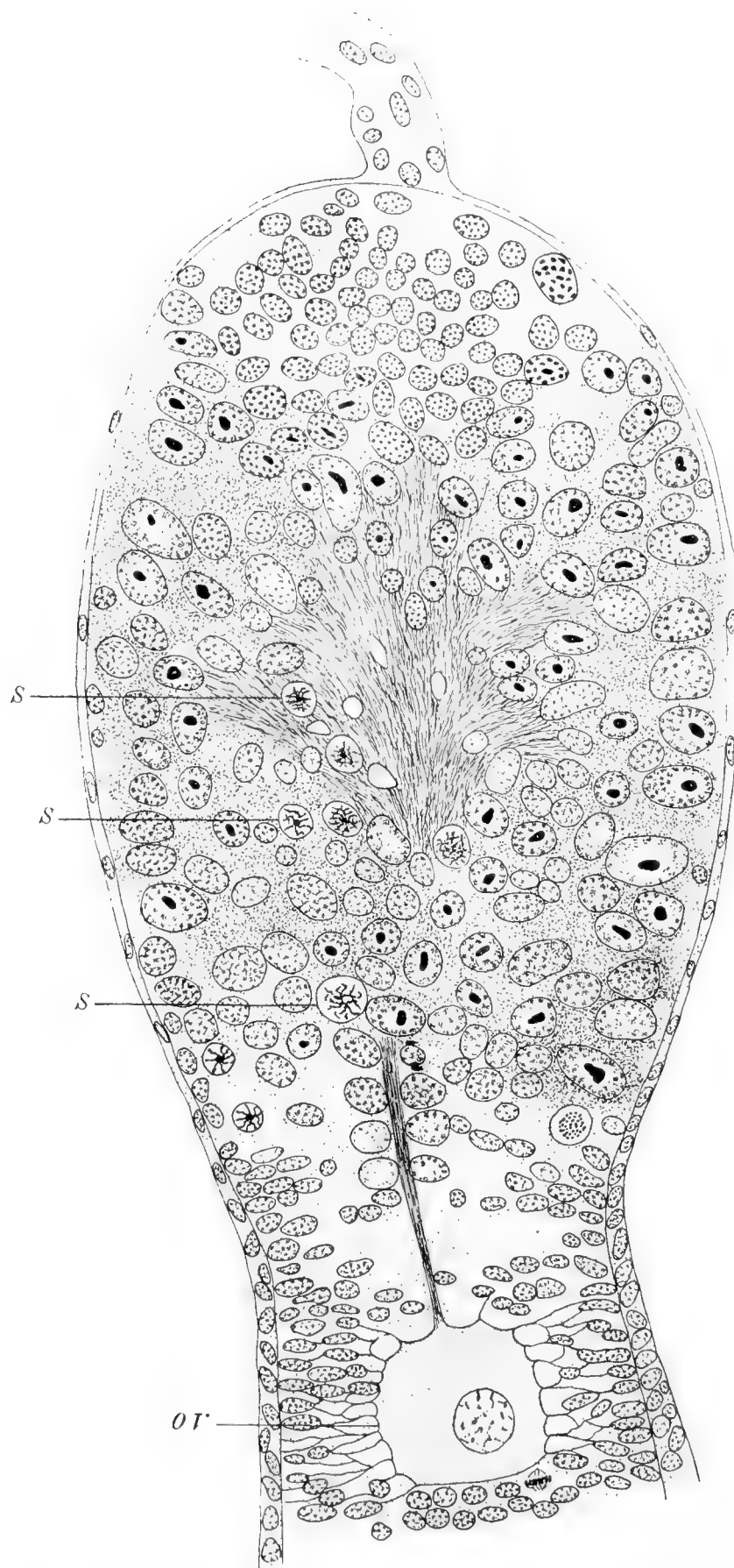


Fig. 10 *Gelastocoris oculatus* Fabr. A longitudinal section through the terminal chamber of a tube from an ovary in which there were eggs approaching maturity. This section shows young oocytes (*S*) in the synaptic stage and an older oocyte (*OV*) as it starts down the tube. Note the small nuclei aggregated about it. $\times 467$.

be the case in all forms. However, it seems to me that Will, and Foot and Strobell have failed to demonstrate conclusively that the ova arise from the small nuclei of zone *C*. At least I do not believe they have figured uninterrupted stages of transition from one to the other and unless it be actually demonstrated that these small nuclei give rise to developing ova, it matters not so far as theories of heredity and chromosomal continuity are concerned, whether they arise by mitosis or amitosis. Further, I do not believe they have exhausted the possibility of the young oocytes being present among the large nuclei of zone *B*.

BIBLIOGRAPHY

- DELLA VALLE, P. 1909 L'organizzazione della cromatina studiata mediante il numero dei cromosomi. *Archivio Zoologica*, tom. 4, no. 1.
- FOOT, K., AND STROBELL, E. C. 1911 Amitosis in the ovary of *Protenor belfragei* and a study of the chromatin nucleolus. *Archiv für Zellforschung*, Bd. 7, no. 2.
- KORSCHOLT, E. 1886 Über die Entstehung und Bedeutung der verschiedenen Zellelemente des Insektenovariums. *Zeitschr. für wiss. Zool.*, Bd. 43, no. 4.
- MONTGOMERY, T. H. 1910 On the dimegalous sperm and chromosomal variation of *Euschistus*, with reference to chromosomal continuity. *Archiv für Zellforschung*, Bd. 5, no. 1.
- PAYNE, F. 1909 Some new types of chromosome distribution and their relation to sex. *Biol. Bull.*, vol. 16, nos. 3 and 4.
- 1910 The chromosomes of *Acholla multispinosa*. *Biol. Bull.*, vol. 18, no. 4.
- PREUSSE, F. 1895 Über die amitotische Kernteilung in den Ovarien der Hemipteren. *Zeitschr. für wiss. Zool.*, Bd. 54, no. 2.
- WILSON, E. B. 1910 Studies on chromosomes VI. A new type of chromosome combination in *Metapodius*. *Jour. Exp. Zool.*, vol. 9, no. 1.

OBSERVATIONS ON THE LIFE-HISTORY OF TWO RARE CILIATES, SPATHIDIUM SPATHULA AND ACTINOBOLUS RADIANUS

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I. SPATHIDIUM SPATHULA

1. *Introduction*

In his "Histoire Naturelle des Infusoires," Dujardin ('41) first described Spathidium as an organism cylindrical in form, very transparent and marked with 20 to 27 delicate, longitudinal striations; although he speaks of the oblique truncation of the anterior end he evidently observed neither mouth nor cilia in this region. Müller in 1786 had described a form, Leucophrys spathula, which with Enchelys gigas of Ehrenberg, Stein and

Entz, was probably synonymous with the form *Spathidium* discovered by Dujardin.

In the main, Ehrenberg's description tallies with that of Dujardin, differing from it only in the account of the anterior region where he found the truncated edge bordered by distinct cilia. Somewhat later, Perty, making a further study of this form, discovered that these cilia surrounded a distinct mouth.

In "Sur la multiplication des Infusoires Ciliés," Maupas gave a fuller description of this interesting organism, which he called *Spathidium spathula*, placing it in the family Enchelinidae of the holotrichous ciliates, although he considered it intermediate between the Enchelinidae and the Trachelinidae, inasmuch as it possesses a form typical of the first family but shows in the region of the mouth an approach to the structures found in the second family.

In the more primitive forms represented by Holophrys, the mouth, a simple passage in direct communication with the endoplasm, is terminal, but many gradations exist between this condition and that found in *Dileptus* where it is situated at the posterior end of a long narrow lobe. Intermediate conditions are to be found in *Spathidium*, *Enchelys* and *Nassula*; the first two possessing a slit-like opening, subterminal in position at the anterior end; while in *Nassula* the opening is about one-third the length of the body from the anterior end. According to Bütschli this change in position of the mouth has come about by the gradual shifting of this organ toward a ventral side until it has come to occupy a more or less central position.

In the flask-shaped body of *Spathidium* Maupas distinguishes a rounded posterior portion, tapering anteriorly to form an elongated neck, bearing at the truncated tip a slit-like mouth armed with trichocysts. In common with the holotrichous ciliates, the entire surface of the body is covered with cilia of equal length except in the mouth region where they are somewhat longer. In the long axis of the body, Maupas found a more or less sinuous band-shaped nucleus accompanied by numerous micronuclei.

This infusorian was found in February, 1911, in a *Paramoecium* culture which had been brought to the laboratory of the

City College from Van Cortlandt Park. Through the kindness of Dr. Goldfarb, some of the culture was sent to Professor Calkins who identified the form as *Spathidium spathula*. Because of its rarity, the peculiar and interesting nuclear changes shown, Professor Calkins suggested that I make a study of its morphological characteristics, its habits, reproductive phenomena, response to stimuli and the processes occurring in its life-history. I wish to express my gratitude to Professor Calkins for his interest in my work and for his helpful suggestions and criticisms.

Before the material came into my hands, Professor Calkins had kept it under observation for a week or more, experimenting with various food-media; he found that *Spathidium* was extremely sensitive to old bacteria infusions. Although Maupas states that *Spathidium* captures and eats all kinds of small ciliates, as for example, *Cyclidium* and *Glaucoma*, during the entire time I had it under observation, I never saw it paralyze or eat any ciliate except *Colpidium*, in fact it seems impossible to cultivate it without the aid of this particular form. From time to time during the past five months, I have found in the original culture containing no *Colpidia*, a few abnormally small *Spathidia*. That the organisms were not in a healthy condition was shown by their greatly reduced size and the perfectly transparent condition of the protoplasm. I am at a loss to account for the free-swimming individuals in the old culture; it may be, however, that some slight change in the environment proved sufficient to cause the emergence of the encysted forms.

In the rich cultures under observation during the first six months of 1911, if for any reason *Colpidium* became reduced in numbers, *Spathidium* encysted. It was quite possible to recover them within four or five hours by adding to the medium containing the cysts, fresh hay-infusion either with or without an abundance of *Colpidium*. A jar containing the original culture was left tightly covered from January 1 to January 12, 1912, upon which date it was examined. Cysts were abundant but no free-swimming forms. A small quantity of fresh hay-infusion was added and the jar was left uncovered. On January

13 large numbers of very small *Spathidia* were found but no *Colpidia*. Change in environment, therefore, would seem to account for the appearance of the small transparent individuals which have been observed in the original culture from October, 1911, to the present date.

2. Material and methods

While details of *Spathidium* *spathula* can be seen only with a compound microscope, I found the binocular much more convenient to use. From a watch glass containing daughter cells of one individual, three were selected on February 24, 1911, and each transferred by means of a capillary pipette to a separate glass dish. These dishes are 4 cm. square, 8 mm. deep and possess a central, circular, shallow cavity, the capacity of which is about $1\frac{1}{2}$ cc., or 80 drops. I have found these small dishes invaluable for this work because owing to the clearness of the glass and the gradual slope of the depression it is impossible for the organism to get into any part of the dish which cannot be brought into focus. They are also more convenient to handle than depression slides. These glass dishes were kept in a moist chamber, a large glass stender dish 10 inches in diameter and 3 inches deep.

After several trials with tap-water, pond-water and hay-infusion, the latter was adopted as the best medium, prepared according to the method of Calkins ('02) and used when twenty-four hours old. In general, the hay-infusion was used undiluted, but toward the end of the series a few drops of pond-water were frequently added with satisfactory results.

Each morning the number of divisions during the preceding twenty-four hours was recorded and one individual from each line isolated according to the following method: into one of the small glass dishes containing six drops of undiluted hay-infusion and one drop of *Colpidium* culture, a single *Spathidium* was transferred by means of a capillary pipette, care being taken to carry over as little as possible of the old medium. This precaution is necessary since the small quantity of fluid in the depression dish soon becomes turbid with the accumulation of

bacteria and the waste products of *Colpidium*, both of which are detrimental to the well-being of *Spathidium*. After isolating one individual from each line, the rest were kept as reserve stock. Such *Spathidium* stock was kept in Syracuse watch-glasses and although many attempts were made to cultivate it in larger dishes, none met with success, the free-swimming forms encysting in large numbers within twenty-four hours.

All dishes used were carefully washed in hot water and dried. The pipettes were also carefully cleaned, a special one being reserved for the purpose of isolation.

Both living and prepared material were studied. Several fixing agents were tried. A saturated aqueous solution of corrosive sublimate with 5 per cent acetic acid gave the most satisfactory results; the other fixatives led to shrinkage of the cells. In making the total preparations, a quantity of *Spathidia* were transferred with a capillary pipette to a watch-glass containing a small quantity of the fixing fluid. They were then carried over to 70 per cent alcohol and allowed to settle to the bottom of the watch-glass. From this they were transferred to a slide smeared with egg-albumen, the alcohol coagulating the albumen and firmly attaching the ciliates. The slide was next placed for half-an-hour in a strong aqueous solution of picrocarmine and, after destaining with acid alcohol, was carried up through the alcohols to xylol and mounted in balsam. Heidenhain's iron haematoxylin and polychromatic methylen blue were also used for staining but the results were unsatisfactory.

The material destined for sectioning was fixed in sublimate-acetic and carried up to absolute alcohol from which it was transferred to an absolute alcoholic solution of magenta. After fifteen minutes in the stain, it was washed in absolute alcohol. Xylol was added drop by drop to the last alcohol until a quantity equal to the amount of alcohol had been used. The material was then transferred to pure xylol. Considerable difficulty was experienced in imbedding when the paraffin-oven was used, due to overheating the material. In transferring from the first to the second paraffin, which must necessarily be done under the microscope, it is difficult to keep the paraffin at the melting

point until the transfer is made. A method was finally suggested which entirely removed this difficulty. A small quantity of soft paraffin in a watch-glass was melted over the water bath. By means of a capillary pipette, the specimens, either individually or en masse, were transferred from the xylol to the melted paraffin. When a film had formed over the surface of the paraffin, the dish was dropped into cold water. The imbedded *Spathidia*, stained bright red, were readily located with the aid of the binocular. Having melted with a warm needle the paraffin surrounding the specimen, it was transferred to a glass slide which had been previously smeared with dilute glycerine. From a pipette of large bore, melted hard paraffin, sufficient to form a good sized drop or sphere, was squeezed upon it. The hot paraffin melts at once the congealed soft paraffin adhering to the specimen and thus embeds it in a homogeneous matrix. The sections were cut $3\frac{1}{2}$ microns thick and stained with iron haematoxylin.

3. Morphology and physiology

Spathidium spathula, as Maupas describes it, is a slender flask-shaped ciliate, the rounded distended posterior end of which is drawn out anteriorly to form a long greatly compressed neck, obliquely truncated at the tip (fig. 1). The records of Dujardin and Maupas show a great variation in the size of the organism, the former quoting 180μ to 240μ as the ordinary length, while Maupas gives 160μ as the outside limit. Of fifty individuals measured, I found the average length and width to be 110.5μ and 35.2μ respectively; the smallest measuring 73.5μ in length with a diameter of 21 ; the largest 157.5μ in length and 57.5μ in diameter.

Maupas described the body as capable of great extension, having often observed it stretched to five or six times its normal length. In swimming, *Spathidium* sometimes becomes entangled in the zooglea at the bottom of the culture dish and under such conditions it often becomes greatly extended in its efforts to escape, but I have never seen it more than double its length and that only on one occasion.

Extending from end to end of the body are many delicate longitudinal striations, marking the lines of insertion of the cilia. These organs are distributed uniformly over the body and are of equal length except in the oral region where those bordering the mouth are somewhat longer. The body is covered by a thin but firm cuticle which gives it its permanency of form allowing at the same time the greatest flexibility. In section the cuticle is seen as a pale area, bounded by a definite line surrounding the more opaque central portion (fig. 2). The protoplasm immediately underneath the cuticle, constituting the cortical plasm, appears somewhat denser than that toward the center, although there is not a distinct line of demarcation between the two areas. The entire protoplasm is finely granular.

As the animal swims rapidly through the water, rotating on its long axis, the anterior end of the body, which is extremely flexible, is in constant motion, bending upward, downward and from side to side as though feeling its way. Owing to this constant change of position, this region continually presents new aspects, as is shown in sketches of many of the total preparations as well as in figures 13 and 17. The mouth, a narrow slit, sub-terminal in position, is bounded by the thickened edges of the truncated tip. Maupas thought the mouth-opening was limited to the extreme posterior part of the oblique edge, but I have found it to extend the entire length of the truncated end. The nature and extent of the mouth can best be determined during the act of feeding. *Spathidium* is a predatory form, swimming actively about as though in search of prey. The moment the anterior end of the body comes in contact with a *Colpidium*, all motion in the latter ceases. *Spathidium* twists and bends its flexible body until the smaller diameter of *Colpidium* is parallel to the truncated edge; the mouth opens, the thickened lips surround the small ciliate which passes slowly down into the body of the captor, the entire process occupying not more than five minutes.

Small spindle-shaped bodies, which in fixed specimens appear as closely crowded fine lines, are imbedded in the thickened rim of the mouth directly underneath and at right angles to

the cuticle (figs. 3 and 12). Bütschli mentions sixteen or more club-shaped, contractile rods, which Maupas interprets as trichocysts. Without doubt the trichocyst material is located in this region, since *Colpidium*, paralyzed when touched by the anterior end, suffers no evil consequences by contact with any other part of the body. Three artificial methods were used to explode the trichocysts: exposure to osmic acid vapor, treatment with a 2 per cent solution of acetic acid and a solution of methyl green. After treatment with any one of these reagents, in fifty individuals examined, the cilia were found fully extended, the trichocysts being readily distinguished from the long oral cilia inasmuch as they were stiff and straight in appearance, whereas the cilia showed a wavy outline. Mitrophanow in his contribution, "Etude sur la structure, le développement et l'explosion des trichocysts des Paramoécies" describes the presence of small bodies in the region of the nucleus which take the nuclear stain. Among these deeply staining masses he found a variety of forms, ranging from spherical granules to rod-shaped bodies, similar in structure to the typical trichocyst, and although he did not actually observe the extrusion of these particles from the nucleus, he thought it probable that the trichocyst material is of nuclear origin, migrating from the interior of the cell toward the periphery. Here by proper stimulation the cortex contracts, the semi-fluid trichocyst material is forced out, solidifying as it comes in contact with the water. I have seen in *Spathidium* no deeply staining particles, originating in the region of the nucleus, which could be interpreted as developing trichocysts; neither have I found the greatly elongated trichocysts of complicated form described by Meier, Schuberg and Schewiakoff for *Frontonia*, *Paramoecium* and some other ciliates; owing, however, to the paralyzing effect on *Colpidium* following contact with the anterior end of the body, I think it safe to interpret, as trichocysts, the short opaque rods so plainly visible in the thickened rim of the mouth.

The mouth opens into a space, the pharynx, reinforced by parallel thickenings of the cortex as shown in figure 18. Imbedded in the protoplasm are numerous food vacuoles, the contents

in various stages of digestion. In many of the total preparations the body of *Colpidium* is seen practically intact (figs. 23 and 25) while in others the cytoplasmic envelope has disappeared, leaving the macronucleus, with the micronucleus lying in a depression on its surface, surrounded by the endoplasm of *Spathidium* (fig. 30). Other deeply staining bodies, varying greatly in size and number, are scattered throughout the protoplasm. Doubtless they are the remains of food particles which have been subjected during a longer period of time to the action of the digestive ferments. According to Calkins, the more conspicuous granules found in the protozoan cell are formed by the breaking down of the food particles, some of which are directly assimilated while others remain as reserve nutriment. He notes the difference in appearance of the protoplasm of a well-fed and a starved *Paramoecium*; that of the former showing a typical granular structure, that of the latter, the entire absence of granules. This contrast is very marked in *Spathidium*, the protoplasm of a well-nourished individual being densely granular, that of the starved forms appearing as a clear and almost structureless substance.

At the posterior end of the body is a large single terminal vacuole which, at room temperature, pulsates on an average of once a minute. Müller describes two vacuoles in *Enchelys spathula*, one near the middle of the body, the other at the posterior end. In the species under observation, two vacuoles are sometimes seen for a time at the posterior end, but sooner or later the two coalesce in one large terminal vacuole (fig. 27). At the systole the contents of the vacuole are expelled through a minute opening at the extreme tip of the body.

Although total preparations and sections of vegetative cells as well as early and later division stages have been carefully studied, the observations have yielded no positive evidence of a differentiation of the nuclear material into two structures, a micronucleus and a macronucleus. In a few instances, vesicular bodies, varying from spherical to retort-shape containing deeply staining granules, have been observed (figs. 10 and 14). The contained granules are elongated and constricted at the center

as though in process of division (figs. 11 and 15); but since these bodies occur so rarely, they cannot be reasonably considered permanent cell structures. The macronucleus, extending lengthwise of the cell, is a greatly elongated, rod-shaped body, circular in section, measuring from 4μ to 8μ in diameter. It is coiled, twisted and folded upon itself, often attaining a length two or three times that of the body (figs. 1, 4 and 5). Although the typical nucleus is long and intricately coiled, a few exceptions were found, two of which are shown in figures 9 and 16. The former might be interpreted as indicative of beginning division; this however is not the case, since in the many division stages studied, the nucleus could always be traced as a continuous band from the anterior to the posterior cell. The macronucleus is bounded by a very delicate membrane which may be readily seen in total preparations which have been compressed by the cover-glass. Here the macronucleus, free from the surrounding protoplasm, retains its definite outline. The chromatin of the macronucleus consists of a mass of minute granules, which take an intense color in staining with iron-haematoxylin. Surrounding the chromatin masses is a faintly staining substance which in section appears somewhat more opaque than the protoplasm of the cell. The granules in the late stages of division are exceedingly fine (figs. 21 and 22). In earlier stages, where an elongation of the cell body has taken place, but as yet no constriction can be seen, the chromatin appears as deeply stained fine threads which would seem to indicate a division of the larger chromatin granules (figs. 19 and 20). Although it is generally thought that the possession of the two types of nuclei is characteristic of the ciliates, the present observations give no evidence of this differentiation in *Spathidium*.

4. Reproduction

Spathidium multiplies by simple fission, the rate of division, according to Maupas, being one division in twenty-four hours. The cultivation of *Spathidium* during two hundred and eighteen generations, showed considerable variation in the division rate. During one ten-day period, the average daily rate of division

was 3.1, the lowest rate of division for the same series for a similar period of time being 0.2 per day. Averaging the high and low division rates of the three lines from February 24 to July 7, 1911, a division rate of 1.5 in twenty-four hours, was found.

Division of the cell and macronucleus is preceded by their gradual elongation, this continuing until the cell has nearly doubled its length, in fact in some cases the dividing cell exceeds twice the length of the vegetative cell.

The variation in form of the macronucleus in division is interesting. Sometimes it is simply folded upon itself, appearing like a single strand except at the point where the two ends separate (figs. 23, 24 and 25); again two distinct pieces may be distinguished, each one folded upon itself and the parts intertwined (fig. 26). Sometimes an exaggerated elongation of the nucleus occurs as shown in figure 27, in which it is extremely difficult to follow the coils and intertwinings.

After the elongation of the cell is accomplished a constriction occurs half-way between the anterior and posterior ends, a new vacuole appearing anterior to this infolding. A little to one side of the point of attachment of the dividing cell, a new mouth is formed in the posterior cell. The position of the new mouth is best seen in the living organism immediately after division. The constriction increases until the cell and the nucleus are divided into approximately equal parts as shown in figures 29, 30 and 31, although an occasional exception is found where the larger part of the nucleus lies in the anterior cell (fig. 28).

During the process of division *Spathidium* swims slowly about, but during the later stages its activity increases. It not only swims more rapidly but it twists the anterior end so energetically that the connection between the two cells is reduced to a delicate strand, this condition continuing for an hour or more. When the slender thread of tissue is severed, the anterior cell swims rapidly away, leaving the posterior half rotating slowly. The posterior individual is at first ovoid in form, the anterior end marked by the new mouth placed somewhat obliquely, the posterior end by the original vacuole. Soon the rotary motion ceases; the new cell swims slowly about, increasing gradu-

ally in length, while the anterior region is drawn out to form the narrow, compressed neck truncated obliquely at the tip.

Although cultures of *Spathidium* were kept under observation from February 24 to July 7 through two hundred and eighteen generations, no conjugation was observed. Attempts were made to bring about this phenomenon by transferring numbers of *Spathidium* to small dishes according to the method of Calkins ('04) but without success.

5. *Encystment*

Encystment takes place either for the purpose of protection against conditions adverse to the well-being of the organism or for the purpose of reproduction. During a period of one month attempts were made daily to cultivate *Spathidium* in dishes of greater capacity than the Syracuse watch-glasses. Staining dishes 3 cm. in diameter and 2 cm. in depth proved fairly satisfactory for a short time, very few encystments occurring during the first two or three days. But although given a sufficient amount of hay-infusion and fed with a rich culture of *Colpidium* at twenty-four-hour intervals, every individual had encysted at the end of the fourth day. All attempts to cultivate *Spathidium* in dishes larger than those described resulted in encystment within twenty-four hours.

To show the process of encystment the following experiments were tried. On March 24, 1911, stock in good condition was transferred to four Syracuse watch-glasses, some of it being kept as control. To all four dishes was added an equal amount of hay-infusion prepared twenty-four hours previously. With a pipette a small quantity of the *Colpidium* culture was transferred to watch-glass *A*. To watch-glass *B* a quantity of *Colpidium* equal in amount to the culture of *Spathidium* transferred was added. In both cases cysts were formed within twenty-four hours. To watch-glass *C* and *D* a medium quantity of *Colpidium* was added. Culture *C* was left over night close to the window where the temperature was considerably below room temperature. In the morning every individual was encysted. Culture *D* was placed on the edge of the table near the radiator at 8 o'clock

in the morning. At noon one-half of the number had encysted. The control, supplied with a few Colpidium remained in good condition, no cysts being found.

The cause of the encystment of the individuals in *A* may be traced to a too small quantity of Colpidium plus an excess of hay-infusion, previous experience having shown that Spathidium will not flourish in a large amount of fluid. Encystment in *B* was due to an excess of the products of Colpidium metabolism the same result having been observed in smaller cultures of Spathidium under similar conditions. Unfavorable temperature conditions probably caused the encystment in *C* and *D*.

By the addition of fresh medium it is often possible to recover Spathidium from the cysts. At 8 o'clock one morning, hay-infusion and Colpidium stock were added to the medium containing encysted forms. The cysts were semi-transparent, the vacuole being distinctly visible. At 9.30 the contents of the cysts began to rotate. After half-an-hour, the form of the body was distinguishable. As the rotation continued, the wall of the cyst became more and more transparent and at 12.45, a portion of the protoplasmic contents was extruded beyond the cyst wall. Five minutes elapsed from the time the first portion of the cell-body appeared to the extrusion of the entire protoplasmic mass.

Upon first emerging from the cyst, Spathidium is weak and shrunken in appearance. The individual under observation remained quiet in the neighborhood of the cyst for several minutes; then swimming slowly about it paralyzed and swallowed a Colpidium within ten minutes of its emergence from the cyst. Figures 35, 36, 37 and 38 give a few of the stages described, the individual being under constant observation from 8 A.M. until 1 P.M.

6. Observations on the life-history

From a culture of Spathidium started on February 24, 1911, three lines, daughter-cells of one individual, were isolated. The average number of divisions daily of the three lines during the first ten-day period was 1.6. This was followed by a slight increase in division rate (1.7) during the second ten-day period.

In the interval between March 13 and March 23, there was a sudden and marked increase, the number of divisions averaging 3.1 per day. Diagram 1 was made according to the method of Calkins. The curve represents the general vitality of the three lines through 218 generations, from February 24 to July 7, 1911. Woodruff defines a rhythm as "a minor periodic rise and fall in fission-rate due to some unknown factor in cell metabolism from which recovery is autonomous" and in summing up his paper on "Rhythms in the reproductive activity of Infusoria" he concludes "that it is not possible by constant environmental conditions to eliminate the rhythms and resolve the graph of the multiplication into an approximately straight line." *Spathidium*, cultivated under constant environmental conditions except for slight variations in the temperature, showed distinct rhythmic fluctuations as indicated by the curve in diagram 1, which result is in close accord with those obtained by Gregory ('09) in a study of the life-history of *Tillina magna*. The decrease in fission rate, followed by the death of the series; may have been due to a difference in the salt content of the water. From February 24 to June 6 Croton tap-water was used, but from the latter date to July 7 spring water was substituted. After March 23 there occurred a sudden decrease in the vitality followed by high division rate from April 2 to April 30. From this time on there was a gradual trend downward as indicated in the diagram.

On June 6, the entire stock was in poor condition as indicated by the low division rate. On this date the individuals were transferred to fresh hay-infusion to which *Colpidium* in abundance had been added. *Spathidium* was sluggish and although it continued to feed, it showed no improvement. Stimulation with beef-extract was tried. At the end of an hour, one out of six had died, and those which survived showed little improvement. At the end of the second hour these individuals showed a return to their normal condition and were transferred from the beef-extract to tap-water then to hay-infusion containing a few *Colpidia*. Although the individuals were normal in appearance, the division rate remained low.

On June 17, the cultures were carried in small phials from New York to Maine. There was no opportunity to examine them again until June 19 when it was found that all except four individuals had encysted and all efforts to force them to emerge from the cysts were fruitless. The four surviving individuals were normal in appearance and continued to divide. On July 2, the culture was in a flourishing condition when without any apparent cause, abnormal forms appeared, many died, while among the surviving individuals the division rate was practically nil. Various stimulants which previously had produced beneficial results, as for example, beef-extract, spring water, $\frac{N}{10}$, $\frac{N}{100}$, $\frac{N}{1000}$ solutions of KCl had no effect. On July 6, six individuals only remained, all of which were abnormal (figs 6, 7, 8). On July 7, three of these encysted, the remaining three disintegrating while under observation. During the remainder of the month many unsuccessful attempts were made to recover the encysted individuals and the race died out in the 218th generation.

7. Regeneration

A few experiments were made to test the regenerating power of *Spathidium*, but as these were limited in number and scope, no general conclusions can be drawn from them.

1. At 11.15 in the morning, half-an-hour after division, a *Spathidium* was transferred to a depression slide, placed upon the stage of the microscope. With a sharp scalpel an incision was made at right angles to the long axis of the body as near the middle line as possible (fig. 32). The anterior half formed a new vacuole, swam slowly about and at 12.15 encysted. The posterior fragment rounded up, gradually elongated and assumed normal shape. It was transferred at 12.15 P.M. and although it swam actively about, coming in contact constantly with its prey, no paralysis of *Colpidium* resulted. It would appear therefore that either the trichocysts were functionless or had not yet been formed. If Mitrophanow's theory of the nuclear origin of these bodies be correct it seems reasonable to believe that sufficient time had not elapsed since the cutting for the

development of trichocyst material. On the following day this individual had divided once and continued to do so normally during the week it was under observation.

2. The same experiment was tried ten minutes after division, resulting in the disintegration of both fragments. The failure to regenerate agrees with the results of Calkins in his experiments on *Uronychia* where he found the regenerating power low immediately after division.

3. Another individual was cut six hours after division, the incision being made at right angles to the long axis of the body but anterior to the middle (fig. 33). The anterior fragment rounded up, swam actively about and at the end of twenty-four hours had entirely regenerated, although it was unusually small. By the following day it had reached normal size and divided twice. The posterior fragment also regenerated and divided normally.

4. A fourth individual was cut across the long axis of the body posterior to the middle, six hours after division (fig. 34). The anterior fragment regenerated in twelve hours. The posterior fragment, small and irregular in shape, gradually rounded up, elongated and assumed normal form. After forty-eight hours, it divided and continued to do so normally during the week it was under observation.

8. Summary

(a) *Spathidium spathula* varies in length from 75.5μ to 157.5μ ; in diameter from 21μ to 57.5μ , the average length and diameter of fifty individuals measured being 110.5μ and 35.2μ respectively.

(b) The mouth is an elongated slit, subterminal in position, extending the entire length of the truncated tip.

(c) Imbedded in the thickened rim of the mouth, are minute opaque rods, the trichocysts, which when artificially exploded, are readily distinguishable from the long wavy oral cilia. The trichocysts are used to paralyze *Colpidium* upon which the organism feeds exclusively.

(d) At room temperature, the single, terminal vacuole pulsates at an average of once a minute.

(e) No evidence has been found in *Spathidium* of a differentiation of nuclear material into a macronucleus and a micronucleus. The macronucleus, a greatly elongated cylindrical organ, sinuous in outline and intricately coiled, possesses a delicate membrane within which lie closely crowded chromatin granules, surrounded by a faintly staining substance. Division of the chromatin masses has been observed in sections of early division stages.

(f) Division of the organism is preceded by an elongation of the body and nucleus; a constriction appears half-way between the anterior and posterior ends; a new vacuole forms anterior to the constriction, a new mouth, posterior to it and a little to one side of the median line of the dividing cell.

(g) Encystment takes place under unfavorable environmental food and temperature conditions. The free swimming forms can be recovered by bringing about normal conditions of environment in respect to food, temperature and quantity of liquid.

(h) Conjugation was not observed, although numerous attempts were made to bring it about.

(i) The descendants of a single individual of *Spathidium* were followed through 218 generations extending from February 24, to July 7, 1911, variations in division rate being graphically represented by a plotted curve. This curve, showing the division rate of the protoplasm, illustrates the normal rhythms described by Woodruff.

II. ACTINOBOLUS RADIANS

1. *History*

Stein observed this rare and interesting organism among the filaments of fresh-water algae in standing water. His description, limited to a footnote in the second volume of "*Der Organismus der Infusionsthier*," is as follows:

Diese neue Gattung beruht auf einem merkwürdigen Thiere, welche ich seit mehreren Jahren bei Niemeck ziemlich häufig in stehenden Gewässern zwischen der vielwurzeligen Wasserlinse beobachtete und welches ich *Actinobolus radians* nennen will. Der Körper ist fast kugelig oder umgekehrt eiförmig, am vorderen Pole mit einem kurzen

zitzenförmigen Fortsatz versehen, in dem die enge Mundöffnung liegt, und ringsum mit gleichförmigen Wimpern besetzt. Zwischen den Wimpern stehen zahlreiche fadenförmigen Tentakeln zerstreut, die sich, wie die Tentakeln der Acinetinen beträchtlich verlängern und auch spurlos in den Körper zurückziehen können. Der After und ein grosser kontraktile Behälter liegen am hinteren Körperpole. Der ziemlich lange strangförmige Nucleus ist unregelmässig zusammengekrümmt. Die Gegenwart von Mund und After schliesst unser Thier entschieden von den Acinetinen aus, denen es auf den ersten Anblick sehr ähnlich scheint.

The next recorded observations are those of Entz published in April, 1883. *Actinobolus*, found in abundance by him in June, had two weeks later entirely disappeared, not only from his cultures, but also from the pond from which his material originally came. To the facts recorded by Stein, Entz adds the following details: The mouth, situated at the anterior end of the body at the tip of a papilla-like structure, leads into a funnel-shaped gullet finely striated. These striations he interprets as folds of the cortex, finding here no differentiation into distinct solid rods, homologous to the basket-like structure found in *Chilodon*. The tentacles, extending outward from the entire surface of the body, he found to be of uniform thickness throughout their length, ending generally bluntly, sometimes terminating in a sharp point, but never knobbed. These organs consisted of a homogeneous substance, capable of great extension and contraction, but different from the tentacles of *Suctorina* inasmuch as in their contracted condition the close spiral appearance observed in the latter, was entirely lacking.

Stein ascribed no special function to the tentacles, but Entz, although he states that he has never seen *Actinobolus* swallow an infusorian, often observed the tentacles closely attached to the filaments of *Cladophora* and other algae, the walls of which upon careful examination, appeared to be ruptured at the point of contact with the tentacles. Entz therefore concluded that the tentacles of *Actinobolus* may assist the animal in securing food, by the secretion of a substance from the tip of the organ, thus dissolving the cell-wall of the plant and exposing the contents to the action of the endoplasmic core of the tentacles.

As a result of these observations he considered *Actinobolus* an holophytic form.

Entz noted a difference between the nuclei found in old and young cells; the nucleus of the former he described as an elongated, cord-like organ, frequently broken up into spherical segments, presenting the appearance of a mass of free nuclei; the nucleus of the latter, a kidney-shaped, horse-shoe form or spherical mass of chromatin. He found, scattered among the filaments of the algae, many thin-walled cysts, within which two, infrequently four, distinct masses of protoplasm were seen rotating; He interpreted the cysts as division or reproductive cysts; the protoplasmic masses as daughter cells. At the time of encystment the following changes occurred: The organism withdrew its tentacles, lost its cilia, the protoplasm became very dense, the nucleus shortened and division of the cell followed. After fission the new individuals emerged from the cyst, taking on within a short period of time, the form of the mature cell, developing tentacles and cilia, the protoplasm again showing the characteristic foamy structure and the nucleus elongating to form the cord-like organ typical of the adult cell.

Von Erlanger, working at Heidelberg under Bütschli, was the next contributor to our knowledge of this ciliate, making a special study of the structure and function of the tentacles. He found these organs regularly arranged in the ciliary grooves extending from anterior to posterior end of the body and could trace them inside of the body in their contracted state, although he states in a foot note that Bütschli was unable to confirm his observation in this particular. In a fully extended tentacle, von Erlanger distinguishes three regions; the proximal part, thick and spherical in form, a long slender portion (both of these parts being perfectly transparent); and a slender opaque rod terminated by a small knob much more minute than the similar structure found in the suctorian tentacle. Fully retracted tentacles formed a peripheral row of minute rods appearing like trichocysts found in other ciliates. Tentacles treated with osmic acid showed an extremely fine dart projecting beyond the knob. Like Entz, von Erlanger never saw the tentacles used to seize

prey, but because of the presence of trichocysts at their tips, he looked upon them as organs of protection.

In 1901 in his paper dealing with some interesting protozoa found in Van Cortlandt Park, Calkins records his observations on the feeding habits of *Actinobolus* and the functions of the tentacles. To quote: ('01b, p. 50)

This remarkable organism possesses a coating of cilia and retractile tentacles which may be elongated to a length equal to three times the diameter of the body, or withdrawn completely into the body. The ends of the tentacles are loaded with trichocysts (Entz '83). When at rest the mouth is directed downward and the tentacles are stretched out in all directions forming a minute forest of plasmic processes, among which smaller ciliates such as *Urocentrum*, *Gastrostyla* etc., or flagellates of all kinds may become entangled without injury to themselves and without disturbing the *Actinobolus* or drawing out the fatal darts. When, however, an *Halteria grandinella*, with its quick jerky movements, approaches the spot, the carnivore is not so peaceful. The trichocysts are discharged with unerring aim and the *Halteria* whirls around in a vigorous but vain effort to escape, then becomes quiet, with cilia outstretched, perfectly paralyzed. The tentacle with the prey fast attached is then slowly contracted until the victim is brought to the body, where by the action of the cilia, it is gradually worked around to the mouth and swallowed with one gulp. Within the short time of twenty minutes, I have seen *Actinobolus* capture and swallow no less than ten *Halterias*.

Thirty-five years, therefore, after its discovery by Stein in 1867, Calkins solved the problem of the function of the tentacles of *Actinobolus*, finding that, contrary to the conclusion of Entz, it is a holozoic form, existing exclusively on the small ciliate, *Halteria*.

2. *Material and method*

The pond water in which *Actinobolus* was found was brought to the laboratory from Van Cortlandt Park. Entz's observation, unverified by Von Erlanger, that the ciliate is always found associated with suctorian forms, was not found to be true in this case, other ciliates and flagellates abounding, but no suctoria. Entz's statement may be accounted for by the fact that *Actinobolus* bears a superficial resemblance to the suctorian *Sphaerophrya* and when at rest might easily be mistaken for this form.

Maupas found bouillon, prepared by boiling a minute quantity of flour in water, a satisfactory medium for the cultivation of infusoria. A modification of this method was adopted for the cultivation of Halteria, but owing to the rapid fermentation of the flour solution it proved unsatisfactory. After experimenting with hay-infusions of various strengths, with tap-water and with pond-water, the latter was chosen as the best medium for the cultivation of Halteria stock. The Actinobolus stock was kept in Syracuse watch-glasses containing pond-water to which had been added a small quantity of Halteria.

Four lines were isolated on October 18, 1911, as subcultures from Professor Calkins' cultures. The same method was employed as for Spathidium culture, Halteria replacing the Colpidium as food. Both living and fixed material consisting of total preparations and sections were studied. Schaudinn's fluid, a mixture of 80 parts corrosive sublimate and 20 parts absolute alcohol, was found to be a most satisfactory fixing agent. The method used in making preparations and sections was the same as that described for Spathidium. Many attempts were made to get satisfactory permanent preparations of the tentacles. Narcotizing with chloral hydrate, killing in Schaudinn's mixture and staining for several hours in either picro-carmin or magenta gave fairly good results. Useful, though not permanent, preparations were obtained by killing in osmic acid vapor and mounting in glycerine. Living material, however, proved far more satisfactory for the study of these organs than any of the fixed preparations described.

3. Morphology and physiology

Actinobolus radians, a holotrichous ciliate, belonging to the suborder Gymnostomina, family Enchelminidae, is a small, almost spherical organism found in fresh water. The average length and diameter of twenty-five individuals measured are 58.5μ and 46.8μ respectively (fig. 39). Regarding the shape of the body Entz and von Erlanger disagree, Entz describing the anterior as the broader region, Erlanger stating that this portion

of the body is tapering. The difference in width between the anterior and posterior ends is slight, but becomes more marked during division when, after the cell has elongated and the constriction appears, the future posterior cell is seen to be of somewhat smaller diameter than the anterior end (figs. 55 and 56).

The anterior region of the body is drawn out to form a minute papilla-like structure, at the extreme tip of which lies the mouth. This projection is not always discernible, however, since this region is extended and retracted at intervals, a slight depression often marking the position of the mouth.

In the living cell two clearly differentiated regions are distinguishable, a dense central region, the endoplasm, surrounded by more transparent area, the ectoplasm. In common with ciliates generally, the body is covered with an extremely thin membrane, the cuticle, the surface of which is marked by 24 or 25 delicate lines extending spirally from the border of the mouth to the posterior end of the body. These lines, which in optical section are seen to be narrow ridges, indicate the places of insertion of the retractile tentacles and the cilia, the latter arranged in small groups at the base of each tentacle. Directly underneath the cuticle is the cortical plasm, a semi-transparent substance in which lie imbedded masses of highly refractive bodies which are in constant circulation. Large vacuoles form a characteristic peripheral border, while strands of endoplasm extend between the vacuoles and grade insensibly into the cortical plasm at the base of the tentacles (fig. 41).

The retractile tentacles, originating in the cortex and capable of an extension equal to two or three times the diameter of the body, are especially interesting. I have already referred to von Erlanger's work on the tentacles of *Actinobolus* in which he distinguishes three distinct regions; a spherical basal portion from which extends a rod-like body bearing at its distal end a sharp dart terminated by a minute knob; the basal sphere and middle portion being perfectly transparent, the dart quite opaque. After treating the animal with osmic acid vapor, he noted an extremely fine thread projecting beyond the knobbed tip of the dart. Although a very careful study has been made of

the organs in total preparations and sections, as well as in the living animal examined under the oil immersion lens, I have been unable to find the structures described by von Erlanger.

With partially retracted tentacles, *Actinobolus*, rotating on its long axis, swims occasionally near the surface of the water, coming to rest from time to time with the mouth downward, on the bottom of the culture dish, moored by means of the oral tentacles. Gradually the retracted tentacles elongate until, seen with the low power, the animal is apparently surrounded by a halo of short opaque rods at varying distances from the periphery of the body. By careful focussing, the opaque rods are seen to be the tips of the tentacles which can be readily traced to the cortical layer of the body. The tentacles are extremely slender organs of equal thickness throughout their length, the tip being slightly rounded, never pointed or knobbed. The opacity of the distal end is due to a mass of minute dark granules, the trichocyst material, which can be seen in circulation as the tentacle is extended.

As shown by Calkins, the tentacles are food-getting organs, *Actinobolus* subsisting exclusively on *Halteria grandinella*; often as many as four or five may be seen attached to these organs, the little ciliates being carried around one by one to the mouth and swallowed. It is a matter of conjecture as to the method by which the capture of this prey is accomplished. Calkins in his description of the action of the tentacles of *Actinobolus* says, ('01a, p. 657):

The trichocyst which von Erlanger described, or its analogue (I was unable to make it out as described by Erlanger), brings down the prey, and the tentacle, by shortening, fetches it to the mouth. The tentacle itself is inserted in *Halteria*, for it can be easily seen with the greatest distinctness when the victim becomes quiet, and I believe that the dart at the end as described is not discharged into the prey but is driven into the soft body of the victim as a minute spear, with shaft attached.

Since the tip of the tentacle is blunt and slightly rounded it seems to me incapable of piercing the cuticle of *Halteria*. In neither fixed nor living material have I found any evidence of a dart-like structure at the tip. The tentacles appear to

me to be fully extended soon after the animal settles down to the bottom of the culture dish, and not shot out on the approach of the prey. This conclusion is based upon many observations of the living animal under the immersion lens, in which I have seen no further elongation of the tentacles at the approach of Halteria. When fully extended, the tentacles are turgid, the ends packed with the dark granular trichocyst material. It seems probable that the impact of the soft body of Halteria against the denser protoplasm of the tentacle is sufficient to drive the latter into the body of the small ciliate, the trichocyst material, forced either through a minute pore or from the ruptured tip, paralyzing the prey. By contraction of the tentacle the Halteria is gradually drawn close to the body of its captor and worked into the mouth by the action of the cilia.

It sometimes happens that the tentacles, stretched out to a great length, become entirely detached from the body. The individual represented in figures 3, 4, 5 and 6 was under observation in a hanging drop for one hour and fifteen minutes. At the beginning of the time it was normal in appearance, swimming actively about; at the end of fifteen minutes it came to rest, mouth downward. Under ordinary conditions the tentacles are extended gradually from the entire surface of the body, but in this case, only three or four were extended, these differing from the normal tentacle inasmuch as they were of enormous length and sinuous in outline (fig. 43). At this stage *Actinobolus* began to rotate very slowly, the extended tentacles, all of great length, increasing in number; then swimming slowly forward, it left in its wake a long trail of cast-off tentacles, many of which showed a worm-like motion (fig. 44). Whether this motion was due to the independent action of the tentacles or to a motion transferred from the cilia to the trailing cluster of detached organs, I am not prepared to say. After swimming about for half an hour, *Actinobolus*, having freed itself of the tangle of liberated tentacles, came to rest. For some minutes the ciliary action was very rapid, but this gradually became slower and finally ceased. Within a few minutes a break appeared on the periphery of the posterior end which was followed

by complete and speedy disintegration of the cell. This phenomenon was observed on three occasions (figs. 45, 46).

Von Erlanger's statement that he was able to trace the partially retracted tentacles for a considerable distance inside the body, I am unable to verify, as I have found no evidence of the presence of these organs beyond the cortical region. Sections of *Actinobolus* show, scattered throughout the endoplasm, many faintly staining threads, some very slender, others comma-shaped, the head of the comma taking the nuclear stain more intensely (figs. 62, 63, 64, 65). Although the threads closely resemble the axial filaments of *Camptonema* pictured by Schaudinn, I have in no case found any connection between them and the nuclear membrane.

Maupas, looking upon the suctorian tentacle as a modified pseudopodium, traced the origin of the ciliates from the rhizopods through this group. Although bearing a resemblance to the suctorian organs, the tentacles of *Mesodinium*, *Ileonema* and *Actinobolus* differ radically from them in structure and function, and, according to Bütschli, appear to be independent modifications rather than organs of phylogenetic interest.

The cilia, arranged in clusters of from five to ten at the base of each tentacle, are long and show a flagella-like motion, rather than the synchronous beating of ordinary cilia.

At the posterior end of the body, situated somewhat eccentrically, is a large contractile vacuole, the pulsations of which occur at intervals of one minute and a half. Once in some thirteen or fourteen beats a minute interval occurs. Surrounding the vacuole is a mass of minute dark granules, waste products of metabolism.

The mouth of *Actinobolus* does not open directly into the endoplasm but is separated from it by a funnel-shaped gullet described by both Entz and von Erlanger, although they do not agree with respect to its structure. Entz noted the striated walls of this organ and attributed the ridged appearance to the presence of folds in the cortex, finding no evidence whatever of solid rods; von Erlanger, on the contrary, interpreted the ridges as distinct straight rods forming a weakly built basket.

The sketches of longitudinal sections through the mouth shown in figures 40 and 42, are typical of the structure of this region. I have found no straight rods; in every case the sections show wavy lines, stained somewhat more deeply than the surrounding protoplasm and having the appearance of cortical thickenings.

A rod-shaped macronucleus is imbedded in the endoplasm which, in different individuals, shows great variety in form, length and position; sometimes lying fully extended and following the circumference of the cell; again straight or loosely coiled in the center of the body; sometimes so tightly twisted as to appear made up of closely united segments (figs. 49, 50, 51, 52). It may have been some such closely twisted form as the latter which Entz described as composed of separate spherical segments, appearing like a mass of free nuclei.

In section the nucleus is seen to be covered with a delicate but distinct membrane, within which, imbedded in an achromatic substance, lie coarse, deeply staining bodies (figs. 42, 47, 53, 65). Scattered throughout the endoplasm are bodies, sometimes spherical, sometimes elongated, many of which are solid masses staining evenly throughout, while others show a differentiation into several intensely staining granules, surrounded by a pale area of definite outline. In one section a comma-shaped body was found made up of distinct granules which appeared to be in process of division (figs. 47 and 48). Similar bodies are shown in figures 54 and 60, sketches of total mounts. It seems impossible from the present observations to determine the nature of these bodies. Although some of them may be micronuclei, I am inclined to interpret them as nuclei of partly digested Halteria, owing to the fact that there is no constancy in number and that none of them has been observed in process of division.

4. Reproduction

Actinobolus radians reproduces by transverse fission, the number of daily divisions showing a wide variation due to the extreme sensitiveness of the organism to food and environmental conditions. During division *Actinobolus* swims actively about,

settling down from time to time to feed; this process which occupies about one hour and a half is preceded by an elongation of the body and nucleus, the difference in width between the anterior and posterior regions becoming more marked as the constriction deepens between the two ends (figs. 54 and 60). The difference in size between the anterior and posterior body is especially well seen when the animal comes to rest mouth downward, the posterior cell resting on top of the anterior one, the peripheries, in optical section, appearing as concentric circles. About half-an-hour after the animal begins to divide, a vacuole is formed anterior to the constriction and a little to one side of the median plane of the body. It is difficult to determine the exact location of the new mouth in the dividing cell, owing to the fact that while the organism is in motion it is impossible to keep it in focus long enough to find the mouth, while, when it comes to rest, this part of the body is directed downward and cannot be seen. When the two cells separate, however, the mouth is readily found at the extreme anterior tip of the body (figs. 55, 56, 57, 58, 59, 61).

5. Summary

(a) *Actinobolus radians* is an almost spherical organism varying in length from 37.5μ , to 77μ , in width from 22.5μ to 66.5μ ; the average length and width of 25 individuals measured was 58.5μ and 46.8μ respectively. It feeds exclusively on *Halteria grandinella*.

(b) Two clearly differentiated regions are distinguishable in the living cell, a dense central mass, the endoplasm, surrounded by a semitransparent envelope, the cortex. Large vacuoles form a characteristic peripheral border.

(c) The cuticle is marked by twenty four or five delicate lines extending spirally from the borders of the mouth to the posterior end of the cell and marking the places of insertion of the retractile tentacles and the cilia.

(d) The cilia are arranged in clusters of from five to ten at the base of each tentacle and show a flagellum-like motion, rather than the synchronous beating of ordinary cilia.

(e) The tentacles, extremely slender organs, are of equal thickness throughout their length; the tip, which is slightly rounded, never pointed or knobbed, is quite opaque, owing to the presence of a mass of minute dark granules, the trichocyst material. The tentacles, greatly extended, are sometimes entirely detached from the body; this phenomenon, in the three cases observed, was followed by speedy disintegration of the organism. These organs, readily traced into the cortical region, have not been observed in the endoplasm.

(f) The single terminal vacuole pulsates at intervals of one minute and a half, a one minute interval occurring once in some thirteen or fourteen pulsations.

(g) The mouth opens into a funnel shaped gullet, the walls of which are strengthened by longitudinal folds of the cortex.

(h) Great variation is seen in form, length and position of the rod-shaped macronucleus; sometimes it lies, fully extended, parallel to the circumference of the body; again, straight, loosely coiled, or tightly twisted in the centre of the cell. The nucleus is covered with a delicate membrane within which, imbedded in an achromatic substance, lie coarse, deeply staining chromatin granules which in division stages are greatly elongated.

(i) Spherical and comma-shaped bodies composed of deeply staining granules imbedded in an achromatic medium, have been observed in many sections and total preparations, which may possibly be micronuclei, but owing to the fact that they have not been observed in process of division, it seems more probable that they are the nuclei of partially digested Halteriae.

(j) *Actinobolus* reproduces by transverse division, the entire process from the first elongation of the cell to the separation of the two individuals, occupying about one hour and a half. The division is unequal, the posterior cell being somewhat smaller than the anterior cell. About half-an-hour after the beginning of division, a new vacuole is formed anterior to the constriction and a little to one side of the median plane of the body. The number of daily divisions shows considerable variation owing to the fact that the organism is extremely sensitive to food and environmental conditions. Encystment was not observed.

III. GENERAL CONSIDERATIONS

A. *The life-cycle, senescence and rejuvenescence*

In 1838 Ehrenberg as a result of his studies of the infusoria concluded that because of their simple structure and method of reproduction, natural death was impossible. This view was opposed by Dujardin who maintained that the life-history shows definite cycles, indicative of successive periods of protoplasmic vitality which eventually end in death.

If the succession of cells formed between two consecutive periods of conjugation is comparable to a metazoan, the study of the cell aggregate, represented in the life-cycle is essential to complete knowledge of the morphological conditions of the species. It often happens in the course of the life-cycle that morphological variations occur, the indications of marked differences in protoplasmic vitality, which, unless the life history has been carefully followed, may lead to confusion in regard to the identification of the forms under observation. For this reason many observers within the last half century have made the life-history of various infusoria the subject of careful study, thereby adding much to our knowledge of these forms.

Among the earlier workers were Bütschli ('76) and Englemann ('78) who were followed by Maupas ('88) the first to make an exhaustive study of the life-cycle. Schaudinn, by his valuable work on *Coccidium schubergi*, emphasized the importance of an intimate knowledge of the life-cycle of every species.

Interesting observations have been made since 1900 on *Paramoecium caudatum* by Calkins ('02 and '04); on *Oxytricha fallax* by Woodruff ('05); on *Gastrostyla mytilus* by Popoff ('07); on *Tillina magna* by Gregory ('08), and on *Paramoecium aurelia* by Woodruff ('09 and '11). With the exception of Woodruff's last work, these infusoria were bred in a more or less constant food medium. As a result of this method of treatment the life-history showed a division into cycles of varying vitality, measured in terms of the division rate. Enriques in his paper of 1908 criticized the results of these experiments and the conclusions based upon them. He denied the existence

of senile degeneration followed by physiological death, claiming that the results obtained by Calkins and Popoff were due to faulty technique resulting in bacterial invasion of the cultures. Calkins' methods, fully given in his paper of 1902, show that a careful technique was followed throughout his work; the cycles which he observed during the 742 generations of *Paramoecium* must therefore have been due to a cause other than the one suggested by Enriques.

Spathidium shows a rhythmic rise and fall in division-rate corresponding to the vitality of the cell and in this respect agrees with the results obtained by Calkins, Woodruff, Popoff and Gregory for *Paramoecium*, *Oxytricha*, *Gastrostyla* and *Tillina*.

Reference to diagram 1 will show that *Spathidium* responded but slightly to treatment with salts. During the period when the division rate fell suddenly from 3.1 to 1.6, the cultures were treated with beef extract. The curve shows only a temporary response, the division-rate gradually dropping to 1.3 during the eighth period.

Artificial stimulation at this time increased somewhat the general vitality, which is indicated by the higher division-rate. That the effect was, however, temporary is shown by the gradual downward trend of the curve, ending in the death of the series. In this respect the life-cycle of *Spathidium* *spathula* closely agrees with the results of Gregory for *Tillina magna* concerning which she says:

Unlike *Paramoecium* and *Oxytricha*, the division rate of *Tillina* does not indicate a definite response to treatment with salts. Such substances, apparently successful in other forms, seem to have been effective only in raising the vitality slightly above the normal and increasing it sufficiently to carry the protoplasm through periods of weakness.

Woodruff defines a cycle as "a periodic rise and fall of the fission rate extending over a varying number of rhythms and ending in extinction of the race unless it is 'rejuvenated' by conjugation or changed environment." The variations in division-rate represented in the life-cycle of *Spathidium* *spathula* may all be interpreted as normal 'rhythms,' the entire curve covering 218 generations, representing but one cycle.

If rejuvenescence mean a renewal of the vital activities of the organisms, this process cannot be said to have taken place in *Spathidium*. In every case artificial stimulation was followed by a slight reaction on the part of the protoplasm, this effect lasting for a short time only, when the protoplasm either lapsed into its original condition or showed a somewhat lower

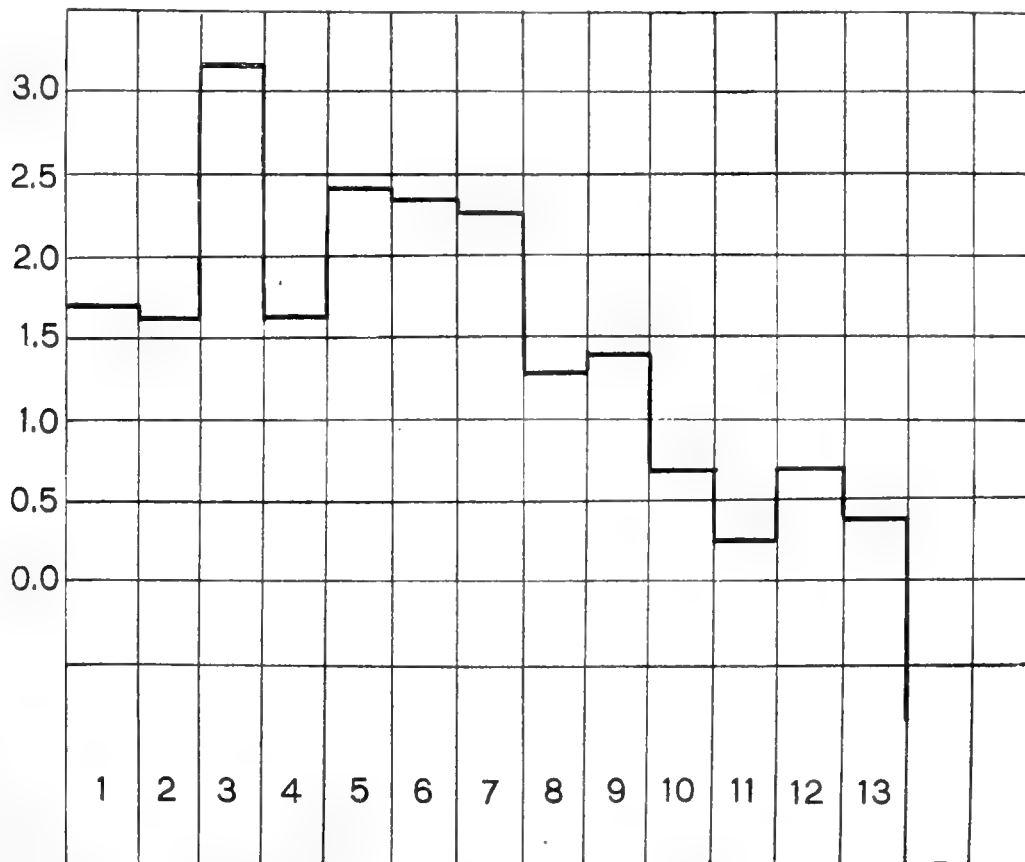


Diagram 1 Complete history of *Spathidium spathula*, Culture A, from start (February 24, 1911) to finish (July 7, 1911) in the 218th generation. The rate of division is averaged for ten-day periods. The ordinates represent the average daily rate of division for three individuals. The abscissae represent the number of ten-day periods.

vitality than before. This response must be interpreted, not as rejuvenescence, in the sense indicated above, but as a temporary response to stimulation, the chemical acting as a spur to the waning activity of the cell. If however we define rejuvenescence as a certain power given to protoplasm by means of which its activities are sustained for a longer period of time

than they would otherwise have been, then to a certain extent *Spathidium* was rejuvenated by the beef-juice, by the treatment of salts, and by the slight change in environment which the cultures experienced when transferred from hay infusion to tap water and later into spring water.

Considerable difference was noted in the amount of reaction manifested by the individuals of the same culture. In some cases there was absolutely no response to treatment with beef extract or salts, while other individuals showed varying degrees of sensitiveness.

If the individuality of the protoplasm of a culture subjected to the same environmental conditions during a long period of time show this marked difference in response to stimuli, it seems reasonable to interpret the effects noted in *Paramoecium*, *Oxytricha*, *Tillina* and *Spathidium* as identical in nature, differing only in degree.

Except during the last period represented in the curve of the life-cycle, *Spathidium* showed no morphological changes coincident with the lowered division rate. Through the first twelve periods of the life history the appearance of the protoplasm was normal; *Colpidium* were consumed and digested in large numbers. It was only at the very end of the series that abnormal forms appeared, this abnormality being accompanied by an extremely dense condition of the protoplasm. Without doubt it would have been impossible to carry the cultures as far as the 218th generation had not the use of beef extract, salts, etc., been resorted to. Since *Spathidium* subsists exclusively on *Colpidium colpoda*, of which it had an abundance, the gradual degeneration of the cultures resulting in physiological death must be traced to a lack in the food medium. The addition of beef extract and salts of various dilutions changed to some extent the chemical composition of the medium and as a result the protoplasm showed a slight response. Owing to the fact that the cultures, aside from a low division energy, showed no signs of depression until the last ten-day period, I am inclined to think the death of the series was due, not to senile degeneration, but to abnormal conditions of environment and that death

might have been avoided or at least averted, had the proper conditions, essential to the well-being of the organism at this crisis, been found.

B. Nucleus-plasma-relation

There has been a growing tendency in recent years to regard the protozoan nucleus as composed of two distinct substances, similar in structure but differing in function; one a trophonuclear matter controlling the vegetative functions of the cell; the other a substance concerned primarily with the reproductive activities of the organism.

The conception of the double nature of the nucleus is due to R. Hertwig, who in 1887 discovered a mass of extranuclear substance in *Arcella*, forming a band between the two vegetative nuclei. In 1889 he observed the formation of secondary nuclei from this extra-nuclear band to which in 1902 he applied the name 'Chromidial-netz.'

We are indebted to Schaudinn ('03) however, for the true interpretation of this chromatin mass. He found that from this extra-nuclear substance, in the case of *Polystomella*, *Centropyxis* and *Chlamydiophrys* minute nuclei are formed which become the nuclei of conjugating gametes. He concluded, therefore, that in these forms the chromidial-netz is sexual chromatin existing in combination with the trophic chromatin during the vegetative condition.

The work of Hertwig and Schaudinn has been greatly extended by Brandt ('02 and '05), Prowazek ('04), Goldschmidt ('05), Lister ('06), Doflein ('07), Calkins ('07), and others. Goldschmidt concluded, as a result of his work on nematodes and protozoa, that every animal cell is binucleate, possessing both trophic and sexual chromatin. These substances are usually combined in one body, the amphinucleus, but the separation of the two elements may be more or less complete.

We may distinguish three distinct degrees of nuclear differentiation among the protozoa; first, a nucleus in which both functions, vegetative and reproductive are combined, the so-

called amphinucleus of Goldschmidt; second, a type, illustrated by Arcella, Centropyxis and others, in which chromatin material is extruded from the nucleus to form the chromidial-netz of Hertwig, or the idiochromidia of Mesnil ('05); third, a type in which there is a complete separation of the two elements into distinct masses, represented by the macro- and micro-nucleus of many infusoria.

Although this complete separation of nuclear material is of common occurrence among the infusoria, it is by no means universal. An examination of the family Encheliniidae in Bütschli's "Protozoa" shows that in the description of fourteen genera, the presence of a micronucleus is positively stated to occur only in the case of Didinium. Among the others, micronuclei have either not been observed or have been little studied. Goldschmidt's opinion that the infusoria show a primitive nuclear condition is opposed by Dobell, who looks upon the complete separation of chromatin substance as another indication of the high specialization shown by these organisms, rather than a simplification as advocated by Goldschmidt. According to Dobell, all functions, somatic and propagative, are resident in the same living nuclear molecule, one or the other predominating in the course of cell differentiation.

It sometimes happens that the separation of the two materials is but temporary. Neresheimer ('08) describes the interesting case of *Ichthyophthirius*, in which the nucleus buds off a smaller nucleus, which divides, each part undergoing two reduction divisions. Three of the resulting micronuclei degenerate. The fourth divides again forming two micronuclei which fuse. The zygote thus formed re-enters the original nucleus and fuses with it. We have here evidently a modification of the first type described, in which both functions are combined in the same body.

Considerable confusion has resulted from the use of the term 'chromidia' applied by Hertwig to the nuclear substance extruded by *Actinosphaerium* when starved or overfed. The chromatin in this case was the direct result of nuclear fragmentation brought about by abnormal conditions in the environment

and was in no sense related to the 'chromidia' of Schaudinn, or the idiochromidia of Mesnil.

I did not find in either *Spathidium* or *Actinobolus* any structures which I felt justified in interpreting as micronuclei. The only bodies which in any way resembled these organs in structure were much too large to be so interpreted. In these organisms both vegetative and propagative chromatin are combined in the long cord-like nucleus, the form and extent of which vary to a remarkable degree. According to Bütschli, the primitive nucleus of the ciliate cell was doubtless spherical, a condition which is found in almost all small ciliates. Using such a type as a starting point it is interesting to follow the evolution of this organ through the ellipsoidal condition, to the short band or rod-shape, on to a greatly elongated nucleus twisted into a complicated coil or divided into numerous ellipsoidal masses enclosed in a common membrane, the increase in length in most cases being coincident with the elongation of the cell body. In 1903 Richard Hertwig first discussed his theory of the nucleus-plasm-relation, maintaining that for each kind of cell in a normal condition, there exists a definite relation between nucleus and protoplasm, upon which the vitality of the cell depends. He called attention to the fact that this normal correlation between cell-size and nuclear-size is destroyed by changes in environment, as for example, an increase or decrease in temperature, starvation or overfeeding, which affect the metabolic activities of the cell controlling the exchanges of nuclear and cytoplasmic material. As a result of this increase of nuclear material, the cell shows symptoms of senile degeneration which end in death unless the normal cell-relations are restored, either by direct elimination of the nuclear material, or by conjugation. In support of his own results, based upon the study of *Actinosphaerium* and *Dileptus* under different environmental conditions, he cited the conclusions reached by Boveri in the case of the sea-urchin larvae and by Gerassimow in his work on *Spirogyra*.

In 1902 and 1905 Boveri studied the relation of cells and nuclei in sea-urchin larvae containing X , $2X$, and $4X$ numbers

of chromosomes, the X larvae, the product of artificial parthenogenesis or the fertilization of an enucleated egg-fragment and the 4 X larvae obtained by shaking the eggs soon after fertilization. As a result of the investigation, Boveri formulated two laws based upon the comparative measurements of these larvae which are as follows: "The surfaces of the nuclei are directly proportional to the chromosome number and hence to the chromatin mass," and "The size of the cell in the sea urchin larvae is directly proportional to the chromosome number." Gerassimow ('01 and '02) found that, if in cell-division of *Spirogyra*, the daughter nuclei were caused to remain in one of the new cells, this cell in consequence grew abnormally large, and he concluded, therefore, that the size of the cell is dependent upon the size of the nucleus.

Summing up the results of the work of Gerassimow ('01, '02), of Boveri ('05) and of Popoff ('07), Hertwig in 1908 says:

Das Neue, welche in der Lehre von der Kernplasma-Relation gegeben ist, ist der Gedanke, dass der Massenverhältnis von Kern zu Protoplasma, der Quotient $\frac{k}{p}$, d. h., Masse der Kernsubstanz dividiert durch Masse des Protoplasma, ein gesetzmässig regulierter Faktor ist, dessen Grösse für alle von Kerne beeinflussten Lebensvorgänge der Zelle, von fundamentaler Bedeutung ist.

In this recent discussion of the kernplasma relation he distinguishes two definite periods of nuclear growth occurring in the interval between consecutive conjugations; first, a 'funktionelle-Wachstum,' a period of extremely slow nuclear growth, accompanied by a rapid increase in volume of the plasma, resulting in a disturbance of the normal kernplasma-relation: Second, a 'theilungs-Wachstum', during which there is a rapid growth of nuclear material by which the normal relation between nucleus and plasma is restored, followed by cell division.

Hertwig's conclusions find support in the recent work of Popoff ('09) who, from a series of measurements made of *Paramecium*, found that the increase in size of the nucleus and plasma between two consecutive divisions, measured at intervals of one hour, did not follow a parallel course. During the first

hours of the growth period there was a rapid increase of protoplasm accompanied by a very slow growth of the nucleus, this condition persisting up to within one hour and a half of the next division, when the sudden and rapid growth of nuclear material re-established the normal kernplasma-relation.

Hertwig, in describing the relation of nucleus and plasma in young cells says: "Ich werde im folgenden diesen Zustand der Kernplasma-Relation, mit welchem die Zelle in eine neue Phase ihren Existenz eintritt, die Kernplasma-Norm nennen." He designates by the term 'Kernplasma-Spannung' that period in the life of the cell when the volume of the nucleus departs from the Kernplasma-Norm. With a view to testing the validity of Hertwig's theory, careful measurements have recently been made of nucleus and plasma in *Tillina magna* by Gregory ('08); in sea-urchin larvae by Erdmann ('08); in *Oenothera lamarckiana* and *Oenothera gigas* by Gates ('09); and in *Crepidula* and *Fulgur* by Conklin('11). Gregory concludes:

These facts seem to prove that there is no relation between the amount of nuclear material in the cell and the general vitality of the protoplasm. In other words, the periods of weakness are not caused by an excess of nuclear material. The nucleus may or may not increase in size during periods of low activity; if an increase does take place, it is generally found that the cytoplasmic material has increased also, and the ratio between the two is the same as in periods of high activity.

Erdmann studied the effect of temperature on the cell size, finding the chromatin volume varied with the temperature and concluding that the chromatin mass and not the number of chromosomes was concerned, and that the cell-size was approximately proportional to this mass. Gates, on the other hand, decided that the larger sized cells of *Oenothera gigas* result from a doubling in the number of chromosomes and not merely from an increase of chromatin mass. Conklin, in summing up the results of his observations on *Crepidula* and *Fulgur* says;

In different eggs, corresponding blastomeres have approximately the same kernplasma-relation; but in different blastomeres of the same egg or of different eggs the kernplasma-relation is neither a constant nor a self regulating ratio. It appears to be a result rather than a cause of the rate of cell-division and consequently a variable rather than a constant factor.

In order to discover and analyze the nucleus-plasma relations existing at different periods of the life-history of *Spathidium* and *Actinobolus*, I made measurements of many cells, in different stages of cell growth, which had been fixed, mounted and stained at different times during the life-history of the organisms. My method consisted in drawing careful outlines of cells and nuclei with the aid of a camera lucida at a magnification of 630 diameters, and then making measurements of the projections. The measurements of lengths and diameters used in the tables are given in microns. In order to obtain a volume of *Spathidium* which was approximately accurate, I used as diameter the aver-

TABLE 1
New cells of Spathidium spathula

	DIAMETER OF CELL	LENGTH OF CELL	DIAMETER OF NUCLEUS	LENGTH OF NUCLEUS	VOLUME OF NUCLEUS	VOLUME OF CELL MINUS VOLUME OF NUCLEUS	KERNPLASMA RELATION
	μ	μ	μ	μ			
1	21	68	6	104	2940.08	20612.4	1:6.1
2	21	92	5	64	1192.96	30672.16	1:25.6
3	22	79	5	79	1472.56	28476.34	1:10.9
4	22	60	6	90	2544.3	20201.7	1:7.9
5	25	60	5	98	1826.72	27625.48	1:15.1
6	27	60	5	55	1025.22	33325.0	1:32
7	26	79	5	190	3541.6	38401.87	1:10
8	22	103	5	158	2945.12	36092.0	1:12.2
9	27	71	6	119	3364.13	37283.4	1:11
10	30	79	6	158	4466.66	52239.6	1:11.6
11	29	63	5	186	3467.04	36117.12	1:10.4
12	27	71	5	143	2665.5	37982.0	1:14.2
13	37	63	6	222	6276.9	61461.3	1:9.7
14	33	69	6	127	3590.2	55425.5	1:15.4
15	20	98	5	87	1621.68	29166.0	1:17.9
16	20	90	5	79	1472.56	26801.84	1:18.2
17	24	82	5	84	1565.76	35498.24	1:22
18	20	68	5	111	2069.04	19293.84	1:9.3
19	27	55	5	95	1770.8	29716.7	1:16.6
20	27	60	5	95	1770.8	32579.2	1:18.3
21	27	63	3	127	897.64	35169.86	1:39
22	31	60	6	174	4918.98	41367.22	1:8.4
	Average	Average	Average	Average	Average	Average	Average
	25 $\frac{15}{22}$	72	5 $\frac{5}{22}$	124 $\frac{17}{22}$	2611.19	34795.85	1:13.3

Average coefficient = 112

age of three measurements taken, one through the thickest region of the cell and two near the extremities. The volume was ascertained by treating both cell and nucleus as cylinders. The formula $\frac{4}{3} \pi r^3$ was used in obtaining the volume of Actinobolus, the cell being treated as a sphere. All volumes are expressed in cubic microns.

In table 1 are given the measurements of twenty-two cells fixed stained and mounted immediately after division. In these new cells which have just begun to nourish themselves and to grow we find, according to Hertwig, in the relation existing between nuclear and protoplasmic volume, the Kernplasma-Norm. An examination of these ratios given in the last column of the table will show a wide variation in the nuclear-plasma relation, ranging from 11:6 to 1:39, giving an average norm of 1:13.3. In table 2 are recorded the measurements of sixteen

TABLE 2
Vegetative-cells of Spathidium spathula

	DIAMETER OF CELL	LENGTH OF CELL	DIAMETER OF NUCLEUS	LENGTH OF NUCLEUS	VOLUME OF NUCLEUS	VOLUME OF CELL MINUS VOLUME OF NUCLEUS	KERNPLASMA RELATION
	μ	μ	μ	μ			
1	35	127	6	157	4438.4	93371.27	1:21.3
2	25	111	5	190	3541.6	50945.97	1:14.3
3	27	127	1.6	286	575.0	132775.0	1:230.9
4	27	132	6	175	4947.25	70623.75	1:14.2
5	24	135	3	292	2064.0	58956.0	1:28.5
6	28	130	5	282	5256.48	74791.02	1:14.1
7	32	114	6	222	62.7594	85407.42	1:13.6
8	19	106	3	95	671.46	29381.66	1:45.7
9	20	132	2	325	1021.02	40448.1	1:30.7
10	41	139	3	301	2127.47	172545.49	1:85.8
11	20	122	2	325	1020.5	37307.02	1:36.5
12	23	111	5	111	2069.04	44048.13	1:21.2
13	29	122	5	238	4436.32	72218.72	1:16.3
14	32	103	3	174	1229.8	81607.92	1:66.3
15	35	99	8	95	4774.7	90474.19	1:18.9
16	29	106	5	214	3989.96	62601.96	1:15.6
	Average 27.5	Average 119. $\frac{5}{8}$	Average 4.1	Average 217. $\frac{5}{8}$	Average 3027.37	Average 74810.23	Average 1:24.6

Average coefficient = 82

vegetative cells taken at random from the cultures. Although a comparison of tables 1 and 2 shows an increase in length and diameter of both nucleus and cell, an examination of the ratios given in the last columns of these tables, indicates that the growth of nucleus and plasma in the vegetative cells has been unequal: that the normal kernplasma-relation has been disturbed in favor of the plasma giving an average ratio of these cells of 1:24.6. A comparison of the average coefficients at these two stages of growth is of interest. This result was obtained by expressing a ratio between the length and diameter of the cell and the length and diameter of the nucleus as for example $\frac{L}{D}:\frac{l}{d}$ in which L and D indicate the dimensions of the cell and l and d those of the nucleus. An increase in size of this resultant indicates an increase in size of the nucleus, a decrease in the value of the coefficient, a corresponding decrease in the size of the nucleus. Comparing tables 1 and 2, we find a decrease in the average coefficient corresponding to the increased kern-

TABLE 3
Division-stages of Spathidium spathula

	DIAMETER OF CELL	LENGTH OF CELL	DIAMETER OF NUCLEUS	LENGTH OF NUCLEUS	VOLUME OF NUCLEUS	VOLUME OF CELL MINUS VOLUME OF NUCLEUS	KERNPLASMA RELATIONS
	μ	μ	μ	μ			
1	24	154	4.7	203	3369.8	75238.2	1:22.3
2	24	157	5	209	3895.76	67068.24	1:17.5
3	22	143	6.3	219	66012.8	47594.2	1:7.2
4	22	143	5	176	3280.0	50916.4	1:15.5
5	30	160	8	277	13922	99175.6	1:7.9
6	29	138	5	206	3839.4	82868.0	1:21.5
7	29	149	5	241	4492.24	89127.44	1:19.8
8	27	143	5	281	5237.8	76629.7	1:14.5
9	24	162	5	582	10848.48	62375.5	1:5.7
10	23	162	3	230	1625.78	65680.37	1:40.3
11	24	169	3	346	2445.52	28742.5	1:11.7
12	24	171	5	222	4027.08	73265.0	1:18.1
	Average	Average	Average	Average	Average	Average	Average
	25 $\frac{1}{6}$	154 $\frac{1}{4}$	5	266	5298.97	68223.43	1:12.8

Average coefficient = 112

plasma-relation on favor of the protoplasm. In table 3 are given the measurements of twelve cells in division stage, the average kernplasma-relation 1:12.8 showing a return to the kernplasma-norm, the average coefficients recorded in tables 1 and 3 being identical. An analysis of the relations existing between nucleus and protoplasm in new cells, vegetative cells, and division-stages show, first, that the nucleusplasma-norm expressed by the ratio 1:13.3 is destroyed during the period of time immediately following division, the ratio between the nucleus and protoplasm being almost doubled in favor of the latter: second, that in the division stages the norm is re-established. These conclusions based on average measurements, agree in the main with the results of Hertwig and Popoff.

Popoff ('08), in his study of *Frontonia leucas*, in discussing the relation between the depression periods and size variations says, "The animals show during this period a marked decrease in cell size and a disturbed kernplasma-relation in favor of the nucleus." And again, "the researches of R. Hertwig in *Actinosphaerium*, *Dileptus* and *Paramoecium*, those of Calkins on *Paramoecium* and of Woodruff on various hypotrichous ciliates, and my own work in *Stylonychia* proves that the protozoa, through a period of uninterrupted activity, come into a condition in which the nucleus shows an abnormal growth." In regard to Calkins' work, Popoff took for granted that the greatly enlarged nucleus was the cause of the depression. Since Calkins, in his paper of 1904, makes no reference to the kernplasma-relation, Popoff must have drawn his conclusions from the photographs of the cells and did not take into consideration the fact that the cells had not divided for several days and were therefore in an abnormal condition.

In table 4 I have recorded the length, diameter and volume of protoplasm and nucleus of newly divided cells, vegetative cells and division stages chosen from two periods in the life-history of *Spathidium*. The cells represented in the first column were taken from the cultures during a ten-day period in March when the division energy was high, averaging for the period 3.1. The figures of the second column are measurements of

cells selected during a ten-day period in May when the division-rate had fallen to 1.3 for this interval. If the division energy be an index of protoplasmic vitality, the cultures in May must have been in a less healthy condition than in March. From May to the death of the series, there was a general decrease in division-rate, averaging but 0.4 during the last ten-day interval in July. Reference to table 4 shows that in May, when the division energy was low and the cultures had already entered on a period of depression, both the coefficient and kernplasma relation indicate a decrease in nuclear volume, rather than the abnormal nuclear growth claimed by Popoff in his paper of 1908.

TABLE 4
Spathidium spathula

	NEW CELLS		RESTING CELLS		DIVISION STAGES	
	March	May	March	May	March	May
Average diameter of nucleus.....	μ 5 $\frac{1}{3}$	μ 4 $\frac{7}{8}$	μ 4.3	μ 4.3	μ 5 $\frac{1}{4}$	μ 4
Average diameter of cell.....	23	24 $\frac{1}{2}$	28	38	23	24
Average length of nucleus.....	81 $\frac{2}{3}$	107	W220	216	202	345
Average length of cell	69 $\frac{5}{6}$	72	W126	117	149	166
Coefficient.....	193	134	83	61	103	80
Kernplasma-relation..	1:14.6	1:15.4	1:24.4	1:43	1:14.4	1:16

Division rate in March = 3.1
Division rate in May = 1.3

Hertwig maintained that an increase in nuclear mass led to a slowing of the division rate. Table 4 shows that this is not true of *Spathidium*, where a slow division-rate is coincident with a decrease of nuclear material.

A careful study and comparison of the nucleus plasma-relation of individual cells shows a wide variation at all periods of the life-history; for example, the measurements of one cell, fixed during March, when the division-rate for the period was 3.1, give a kernplasma-ratio of 1:230 in favor of the protoplasm, also the average of ratios during a period of low division energy

indicates a reduced nuclear volume. On the other hand, cells with a ratio very close to the kernplasma-norm were abundant during periods of reduced division energy. The variations noted in *Spathidium* are seen by consultation of table 5, to be also true for *Actinobolus*. These facts therefore, seem to indicate that the kernplasma-relation is not a constant quantity and bears no definite relation to the division-rate. In spite of the wide departure from the kernplasma-norm shown in the vegetative cells recorded in table 2, this divergence, resulting in a disturbed kernplasma-relation has not resulted in division of the cell. It seems therefore that division in *Spathidium*

TABLE 5
Vegetative-cells of Actinobolus radians

	DIAMETER OF CELL	DIAMETER OF NUCLEUS	LENGTH OF NUCLEUS	VOLUME OF NUCLEUS	VOLUME OF CELL MINUS VOLUME OF NUCLEUS	KERNPLASMA RELATION
	μ	μ	μ			
1	47	6	143	50965	46922	1:11.6
2	47	6	119	50965	47601	1:14.1
3	39	6	135	28730	24914	1: 6.5
4	55	6	87	82448	79989	1:32
5	47	6	143	50965	46922	1:11.6
6	39	5.5	119	28730	26112	1:10
7	39	4.7	150	28730	26075	1:9.8
8	39	4.7	142	28730	26501	1:11.8
9	67	6	174	150532	145617	1:29.5
10	55	5.5	174	82448	78620	1:20.5
11	31	3	87	14137	13528	1:22
12	47	6	95	50965	48279	1:17.9
13	43	4.7	103	38792	37175	1:22.9
14	71	4.7	174	179595	176863	1:64.7
15	55	6	190	82448	77077	1:14
16	39	6	95	28730	26049	1:9.7
17	63	6	206	124789	119505	1:20.7
18	55	4.7	135	82448	80329	1:37.7
19	39	4.7	135	28730	26611	1:12
20	63	4.7	135	124789	122670	1:57.8
21	39	4.7	103	28730	27113	1:16.7
22	47	4.5	158	50965	47489	1:13.6
23	35	4.5	111	20580	18138	1:7
24	55	6	166	82448	77755	1:16.5
25	47	4.5	166	50965	27313	1:7.5

Average kern-plasma relation = 1:20.

cannot be traced to a disturbed relation between nucleus and protoplasm. These varying ratios found in cells of the same period in the life history of the organism, may be explained on the basis of differences in cell metabolism. Cultures subjected to comparatively constant environment showed wide individual differences in response to artificial stimulation and it seems probable that the variations in the kernplasma-relation are individual variations due to a difference in response to environmental conditions, since the interchange of nucleus and protoplasmic material is dependent upon the metabolic activity of the cell. Conklin states in his paper on "Cell size and nuclear size" that "As we have seen, the kernplasma-relation varies widely in different blastomeres of *Crepidula* and *Fulgur*. In these cases wide departures from the kernspasma-norm have not brought on cell division and if the kernspannung is a cause of cell-division, it must be a minor factor in the case." From the evidence at hand therefore it seems impossible to trace the phenomena of cell division to any of the causes thus far suggested by Spencer, Strasburger, Boveri or Hertwig. Cell-division is the index of protoplasmic vitality which is directly dependent upon the metabolic activity of the cell. If therefore, we accept Child's interpretation of senescence and rejuvenescence, we must attribute the varying relations between nucleus and protoplasm to individual differences in metabolic activity expressed in the division-rate.

Strasburger ('93) accounts for cell division on the basis of the 'working-sphere' of the nucleus. The experimental work of Brandt ('77), Nussbaum ('84), Gruber ('85), Lillie ('96), Balbiani ('89 and '91) and Verworn ('89) all go to show that the nucleus is indispensable to the formative energy of the cell; that although the processes of destructive metabolism may continue for a long time in an enucleated cell, that constructive metabolism is only possible in the presence of the nucleus.

Considering these facts in connection with Strasburger's theory of the 'working-sphere' of the nucleus, the ratio existing between the nuclear surface and the protoplasmic mass must be of great significance in the interchange of nuclear and protoplasmic

material. The maintenance of a certain ratio between the two masses, making possible a normal interchange between them of material, seems to me to explain the greatly elongated nuclei found in *Spathidium* and *Actinobolus*.

C. Food habits

Frequent observations of the feeding habits of *Actinobolus* and *Spathidium* are suggestive of many interesting questions. In these two forms we have organisms subsisting exclusively, so far as we know, upon a special type of ciliate. *Actinobolus*, moored by means of its oral tentacles, awaits the coming of its victim, *Halteria grandinella*, before making use of its weapons of offense. *Spathidium*, a predatory form, swims actively through the water, its anterior end in constant motion, passing with seeming indifference all food material except the little ciliate, *Colpidium colpoda*. This behavior on the part of the organism would seem to indicate an apparent exercise of choice, but when one comes to a careful analysis of the basis of this choice, one finds himself becoming involved in questions upon which the observations of many other workers have thrown but little light.

Interest in the feeding-habits of unicellular organisms, resulting in practical experimentation dates back to the time of Gleichen in the latter half of the eighteenth century. The question which arose in the minds of these early observers in the field of protozoology was one which has been discussed by almost every worker on the protozoa since that time, namely: Have these simple forms of animal life the power of selection in the kind of food-material upon which they live,—if so, upon what is the choice based? Many of the experiments of Gleichen showing that large quantities of finely powdered carmine or indigo mixed with the water, were taken into the body of these unicellular forms, were repeated and confirmed by Ehrenberg in 1838. Stein, Entz and many other workers, however, either forgot or ignored these early results, since they expressed the concensus of opinion at that time that infusoria showed a decided power of selection, inasmuch as from the mass of sub-

stance swept into the pharynx, food-particles were passed into the endoplasm while all foreign substances were rejected by a reversal of ciliary motion. This question was again taken up by Verworn in 1889 who, after experimenting on *Vorticella* with chalk crystals, carmine and indigo concluded "wenn man die Bezeichnung Auswahl für einzelne dieser Erscheinungen beibehalten will, sich klar machen müssen, dass darunter keine bewusste Auswahl in einer bestimmten Absicht zu verstehen ist, sondern ein völlig unbewusster Vorgang, ähnlich der natürlichen Auswahl, der Selection, die der Kampf um's Dasein hervorbringt."

Hodge and Aiken in 1893 published a paper on the "Daily Life of a Protozoan" with the significant sub-title "A Study in Comparative Psycho-Physiology." Here again *Vorticella* was the subject of the experiment, chosen because it is easily kept in the microscopic field during long periods of time. The fact that the same animal subjected to similar conditions of experiment shows different reactions at the hands of two skillful observers, leading to diametrically opposed conclusions is of interest. Hodge classed the action of the cilia under psycho-reflex movements, assigning to them a sorting or discriminating function, dependent apparently upon a touch sensation and concluded that this process indicated a no less conscious action on the part of *Vorticella* than the seeking of prey and the feeding of animals in general.

Schaeffer, in 1910, published the results of some exceedingly interesting feeding experiments on *Stentor*. Every test was most carefully controlled in order that the exact kind and quantity of food might be accurately recorded. Without going into the details of the experiments I will simply quote his results which are of interest and importance in the light of other experimental work on the feeding habits of Protozoa. Schaeffer found that, as food, *Stentor* preferred *Euglena* to *Phacus*; that it could discriminate between *Phacus triqueter* and *Phacus longicaudus*, also between *Trachelomonas hispida* and *Trachelomonas volvocina*; that it manifested no choice between living organisms and those killed in chemicals, as for example osmic acid, iodine

or alcohol; that whole specimens were eaten while a jelly composed of crushed *Paramoecia* and *Euglena* was rejected. As a logical deduction from these results Schaeffer concluded that *Stentor* does exercise a selection among particles which are brought into the food pouch; it discriminates between digestible and indigestible particles and between different kinds of organisms. What then is the basis of this choice? Making a distinction between taste, a reaction to chemicals, and touch, a reaction to form, he concludes that inasmuch as *Stentor* ate alike living forms and those fixed in chemicals while it chose entire organisms in preference to macerated ones, that the selection must be made on a tactual basis.

Didinium, a predatory ciliate, appears to the casual observer to exercise a somewhat limited choice. Jennings, however, explains the food habits of this form by the trial-error theory, claiming that *Didinium* reacts not only to particles which may serve as food but to all kinds of solid bodies, that it is constantly coming into contact with various substances digestible and indigestible, each one of which it 'tries' to pierce and swallow. If successful the particle is chosen as food, if the trial be unsuccessful it is rejected and classed with the errors. Jennings says "there is no evidence that in some unknown way the infusoria perceive their prey at a distance nor that they decide beforehand to attack certain objects and leave others unattacked. They simply prove all things and hold fast to that which is good." In this conclusion Jennings agrees with Maupas.

It is evident, however, that the trial-error explanation is inadequate in dealing with the behavior of *Actinobolus* and *Spathidium*. It is true that when the organisms are in a condition of satiety, the small ciliates *Halteria* and *Colpidium* may pass their enemies unharmed; if, on the other hand, *Actinobolus* and *Spathidium* are hungry, no attempt is made to eat any other kind of food than the accustomed prey. We find here no 'trials' but a definite selection of food material. In experimentation with unicellular forms under identical conditions not only has a difference in reaction between different individuals been noted, but also difference in reaction of the same individual at differ-

ent times. These reactions may be accounted for by an explanation on the basis of chemical or physical laws of attraction or by assigning to the organism something akin to intelligence. The question naturally suggests itself, how has the exercise of choice become limited to one special form of food material in the case of *Actinobolus* and *Spathidium*? If, as Jennings claims, the avoiding action of the cilia in *Paramecium* is in itself an expression of choice, it is not difficult to conceive of a still further development of this expression as illustrated in the reversal of the ciliary current, the bending of the body of the organism and the swimming away from foreign substances or undesirable food material. Accepting the definition of instinct as purposive action without consciousness of purpose, it seems legitimate to apply the term in this sense to the phenomena involved in the food taking behavior of protozoa. The word thus used is simply a convenient term for expressing the outward manifestation of a relation existing between the protoplasm of an organism and an external stimulus or another form of protoplasm possessed of different chemical or physical properties. It may be that the behavior of the early ancestors of the hunter ciliates was based on the method of trial. In the course of time, the protoplasm of the organism has become modified chemically and physiologically to such an extent that a reaction to one kind of protoplasm only is possible—in other words forms like *Actinobolus* and *Spathidium* have become 'educated' through 'error' to the selection of one species of food, namely, *Halteria grandinella* and *Colpidium colpoda*.

March 9, 1912

LITERATURE CITED

- BALBIANI, E. G. 1889 Recherches expérimentales sur la merotomie des infusoires ciliés. Recueil Zool., Suisse, tom. 5.
1891 Sur les régénérations successive du péristome chez les Stentors et sur la rôle du noyau dans ces phénomènes. Zool. Anzeiger, Bd. 14.
- BOVERI, T. 1905 Zellenstudien V. Ueber die Abhängigkeit der Kerngrösse und Zellenzahl der Ausgangszellen.
- BRANDT, H. 1877 Über *Actinosphaerium* *Eichhorni*.

- BRANDT, K. 1902 Beiträge zur Kenntnis der Colliden I und II. Archiv für Protistenkunde, vol. 1.
- BÜTSCHLI, O. 1883 Protozoa. Bronn's Klassen und Ordnungen des Thier-Reichs.
- CALKINS, G. N. 1901a Some Protozoa of especial interest from Van Cortlandt Park, New York. Am. Naturalist, vol. 35.
- 1901b The Protozoa. Columbia University Press.
- 1902a Studies on the life-history of Protozoa. II. The effect of stimuli on the life-cycle of *Paramoecium caudatum*. Archiv für Protist.
- 1902b III. The six hundred and twentieth generation of *Paramoecium caudatum*. Biol. Bulletin, vol. 3.
- 1904 IV. Death of the series: Conclusions. Jour. Exp. Zool. 1.
- 1906 The protozoan life-cycle. Biol. Bulletin, vol. 10.
- 1907 The fertilization of *Amoeba proteus*. Biol. Bul., vol. 13.
- 1910 Protozoölogy.
- 1911a Regeneration and cell-division in *Uronychia*. Jour. Exp. Zool. vol. 10.
- 1911b Effects produced by cutting *Paramoecium* cells. Biol. Bull., vol. 21.
- 1911c The scope of protozoology. Science, vol. 34.
- CHILD, C. M. 1911 A study of senescence and rejuvenescence based on experiments with *Planaria dorotocephala*. Arch. F. Entw.-Mech., Bd. 31.
- CONKLIN, E. G. 1912 Cell size and nuclear size. Jour. Exp. Zool., vol. 12.
- DOBELL, C. C. 1909 Chromidia and the binuclearity hypothesis. Q. J. Mic. Sc., vol. 53.
- DOFLEIN. 1907 Fortpflanzungserscheinungen bei Amöben und verwandten Organismen. SB. Ges. Morph. Physiol. München, Bd. 23.
- DUJARDIN, F. 1841 Historie naturelle des Infusoires.
- EHRENBERG 1838 Die Infusionsthierchen als vollkommene Organismen.
- ENRIQUES, P. 1908 Die Conjugation und sexuelle Differenzierung der Infusorien. Archiv f. Protistk, vol. 12.
- ENTZ, G. 1879 Über einige Infusorien des Salzteiches zu Szamosfalva.
- 1883 Beiträge zur Kenntnis der Infusorien. Zeitsch. f. wiss. Zool., Bd. 38.
- VON ERLANGER 1889 Zur Kenntnis einiger Infusorien. Zeitsch. f. wiss. Zool., Bd., 49.
- ERDMANN, RH. 1909 Experimentelle Untersuchung der Massverhältnisse von Plasma, Kern und Chromosomen in dem sich entwickelnden Seeigeli. Arch. f. Zellforschung, Bd. 2.

- GATES, R. R. 1909 Stature and chromosomes of *Oenothera gigas*. Arch. f. Zellforschung, Bd. 3.
- GERASSIMOW 1902 Die Abhängigkeit der Grösse der Zelle von Menge ihrer Kernmasse. Zeitsch. f. Allgem. Physiol., Bd. 1.
- GOLDSCHMIDT, R. 1905 Die Chromidien der Protozoen. Arch. f. Prot., vol. 5.
- GREGORY, L. H. 1909 Observations on the life-history of *Tillina magna*. Jour. Exp. Zool., vol. 6.
- GRUBER, A. 1885 Über künstliche Teilung bei Infusorien. Biol. Centralblatt, vol. 5.
- HERTWIG, R. 1887 Über die Kernteilung der Infusorien. SB. Ges. Morph. Physiol. München.
- 1899 Über Encystierung und Kernvermehrung bei *Arcella vulgaris*. Festschrift f. Kupffer.
- 1902 Die Protozoen und die Zelltheorie. Arch. f. Protistk., vol. 1.
- 1903 Über Korrelation von Zell- und Kerngrösse und ihre Bedeutung für die geschlechtliche Differenzierung und die Teilung der Zelle. Biol. Centralb., Bd. 22.
- 1908 Über neue Probleme der Zellenlehre. Arch. f. Zellforsch., Bd. 1.
- JENNINGS, H. S. 1898 The psychology of a protozoan. Am. Jour. of Psych., vol. 10.
- 1902 Studies on reactions to stimuli in unicellular animals. Am. Jour. of Physiol., vol. 8.
- 1906 Behavior of the lower organisms.
- LILLIE, F. R. 1896 On the smallest parts of *Stentor* capable of regeneration. Jour. Morph., vol. 12.
- LISTER, J. J. 1906 The life-history of the Foramenifera. Pres. Add. Sec. D. Brit. Assoc.
- MAIER, H. N. 1903 Über den feinen Bau der Wimperapparat der Infusorien. Arch. f. Protistenk., vol. 2.
- MAST, S. O. 1909 Reactions of *Didinium nasutum* with specific reference to the feeding habits and the functions of trichocysts. Biol. Bull., vol. 16.
- MAUPAS, E. 1888 Recherches expérimentales sur la multiplication des infusoires ciliés. Arch. de Zool. Expér. et Gen.
- 1883 Contribution à l'étude morphologique et anatomique des infusoires ciliés.
- MESNIL, F. 1905 Chromidies et questions connexes. Bull. Inst. Pasteur.
- MINOT, C. S. 1908 Age, growth and death.
- MITROPHANOW 1905 Étude sur la structure, le développement et l'explosion des trichocysts des Paramécies. Arch. f. Protistenk., Bd. 5.

- MÜLLER, O. F. 1786 *Animalcula infusoria*.
- NERESHEIMER, E. 1908 Der Zeugungskreis des *Ichthyophthirius*. Ber. bayer. Biol. Versuchungstation. München.
- NUSSBAUM, M. 1884 Über Spontane und Künstliche Theilung von Infusorien. Vehr. d. natur. Ver. preus. Rheinland.
- PERTY, J. A. 1852 Zur Kenntniss kleinster Lebensformen.
- POPOFF, M. 1907 Depression der Protozoenzelle und der Geschlechtszelle der Metazoen. Arch. f. Prot., Bd. 1.
- 1908 Experimentelle cytologische Studien. Arch. f. Zellforschung, Bd. 1.
- 1909 Experimentelle Zellstudien
- II. Über die Zellgrösse, ihre Fixierung und Vererbung. Arch. f. Zellforsch., Bd. 3.
- PROWAZEK, S. 1904 Untersuchungen über einige parasitische Flagellaten. Arb. Kaiserl. Gesundheitsamte.
- SCHAEFFER, J. P. 1910 Selection of food in *Stentor*. Jour. Exp. Zool., vol. 8.
- SCHAUDINN, F. 1896 *Camptonema mutans*. Sitz. Ber. k. preuss. Akad. Wiss. Bd. 53.
- 1903 Untersuchungen über die Fortpflanzung einigen Rhizopoden. Arb. kaiserl. Gesundheitsamte.
- SCHEWIAKOFF, W. 1889 Beiträge zur Kenntniss der holotrichen Ciliaten. Bibliothek zool., Heft. 5.
- STEIN, F. 1867 Der Organismus der Infusionsthier, vol 2.
- STRASBURGER, E. 1893 Über die Wirkungsphäre der Kerne und die Zellgrösse.
- VERWORN, M. 1889 Protistenstudien.
- 1892 Die physiologische Bedeutung des Kernes. Arch. f. gesamt. Physiol., Bd. 5.
- WILSON, E. B. 1906 The cell in development and inheritance.
- WOODRUFF, L. L. 1905 An experimental study on the life-history of hypotrichous Infusoria. Jour. Exp. Zool., vol. 2.
- 1909 Further studies on the life-cycle of *Paramecium*. Biol. Bull. vol. 17.
- 1911 Two thousand generations of *Paramecium*. Arch. f. Protistenk., vol. 2.
- 1911 Evidence of adaptations of *Paramecium* to different environments. Biol. Bull., vol. 22.
- WOODRUFF, L. L., AND BAITSSELL, G. A. 1911 Rythms in reproductive activity of Infusoria. Jour. Exp. Zool., vol. 11.

DESCRIPTION OF PLATES

Unless otherwise stated, all figures are made from camera drawings or sketches from the living cells.

The figures in plates 1 and 2 are of *Spathidium spathula*, those in plates 3 and 4 of *Actinobolus radians*.

PLATE 1

EXPLANATION OF FIGURES

1 Freehand sketch of *Spathidium* showing nucleus, contractile vacuole, mouth, lines of insertion of the cilia and oral cilia.

2 Optical section of total preparation to show cuticle, cortex, endoplasm and chromatin granules in the nucleus. $\times 400$.

3 Sketch of total mount showing trichocysts in rim of the mouth. $\times 400$.

4 and 5 Sketches of total preparations to show variations in form of the nucleus. $\times 400$.

6, 7 and 8 Freehand sketches of abnormal forms found a short time before the death of the series.

9 Total preparation showing the nucleus divided into two distinct parts each of which is tightly coiled. $\times 400$.

10 Longitudinal section showing fragments of the macronucleus and an elongated vesicular body containing deeply staining bodies in process of division. $\times 415$.

11 A retort-shaped body found in section represented in fig. 14. $\times 1673$.

12 Anterior region of body showing trichocysts, drawn from total preparations.

13 Sketch of mouth region drawn from living form.

14 Longitudinal section showing nucleus, food particles and retort-shaped body shown in figure 11. $\times 415$.

15 Sketch showing relation of the elongated and retort-shaped bodies found in the serial sections pictured in figure 10 and 14. $\times 1673$.

16 Total preparation illustrating another variation in the form of the nucleus. $\times 415$.

17 Sketch of mouth region from living animal.

18 Section showing cortical foldings in the gullet. $\times 415$.

19 and 20 Sketches of early division stage to show elongated chromatin granules. $\times 415$.

21 and 22 Sketches of a late division stage showing the fine chromatin granules. $\times 415$.



F.M. del

PLATE 2

EXPLANATION OF FIGURES

23 to 27 Total preparations of early division stages showing greatly elongated cell and nucleus. $\times 400$.

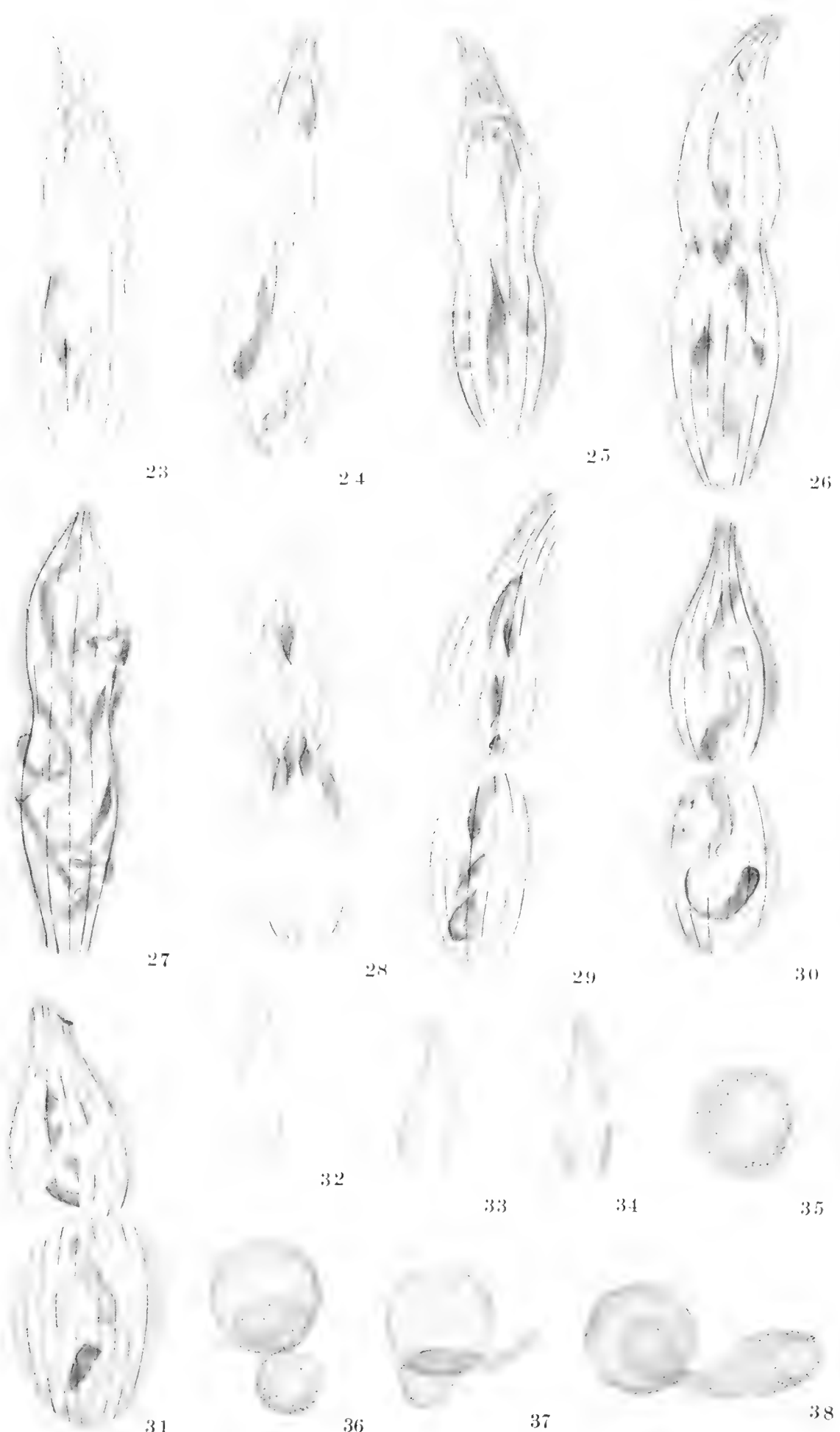
28 to 31 Late division stages illustrating great variation in the size and form of nucleus in the new cells. $\times 400$.

32 Diagram to show the position of the incision made in experiments 1 and 2 in regeneration.

33 Diagram showing position of incision made in experiment 3.

34 Diagram to show place of cut made in experiment 4.

35, 36, 37, 38 Sketches made from the living form while emerging from the cyst.



J. E. M. del

PLATE 3

EXPLANATION OF FIGURES

39 Sketch of living animal showing nucleus, contractile vacuole, tentacles and cilia. Dorsal view.

40 Drawing of section through the mouth region to show the longitudinal folds of the gullet; a partially digested Halteria in a food vacuole and three fragments of the nucleus. $\times 415$.

41 Sketch from living animal. The periphery of the cell drawn under oil immersion lens; to show peripheral vacuoles, cilia, tentacles, cuticle, cortex, endoplasm and refractive bodies. $\times 787$.

42 Sketch of section following the one represented in figure 40. $\times 415$.

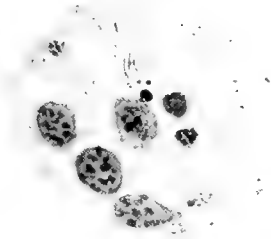
43 Sketch of living cell to show four greatly elongated tentacles.

44 Drawing of same individual swimming and dragging a long trail of detached tentacles.

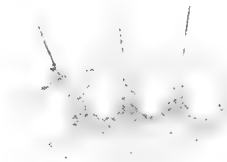
45 and 46 Degeneration stages of the same individual.



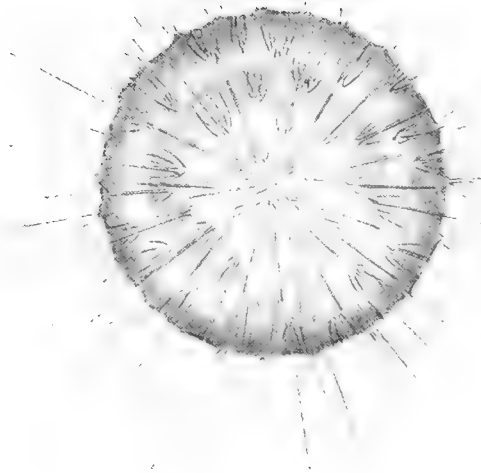
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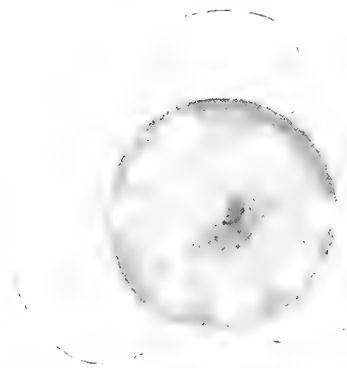
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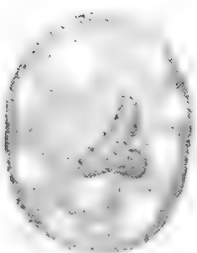
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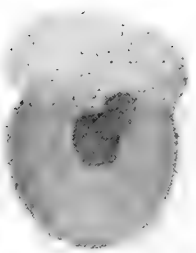
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46

PLATE 4

EXPLANATION OF FIGURES

47 Sketch of section to show two fragments of the nucleus, a retort-shaped vesicular body containing masses of chromatin which appear to be in process of division; and the peripheral vacuoles. $\times 415$.

48 The retort-shaped body shown in figure 47, greatly magnified. $\times 1673$.

49 Sketch of total preparation showing an extended nucleus following the circumference of the cell. $\times 415$.

50 Total preparation to show the loosely coiled nucleus in the center of the cell. $\times 415$.

51 Sketch of a section showing an almost straight nucleus. $\times 415$.

52 Sketch of a total preparation in which the nucleus is seen tightly twisted. $\times 415$.

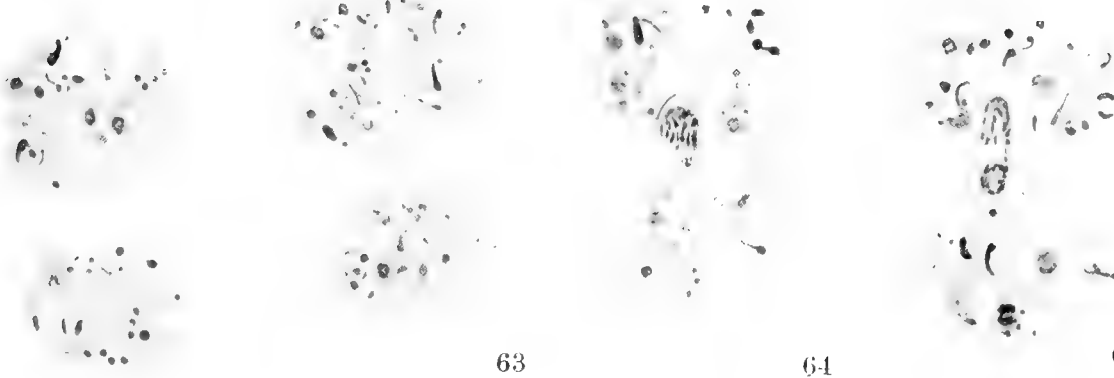
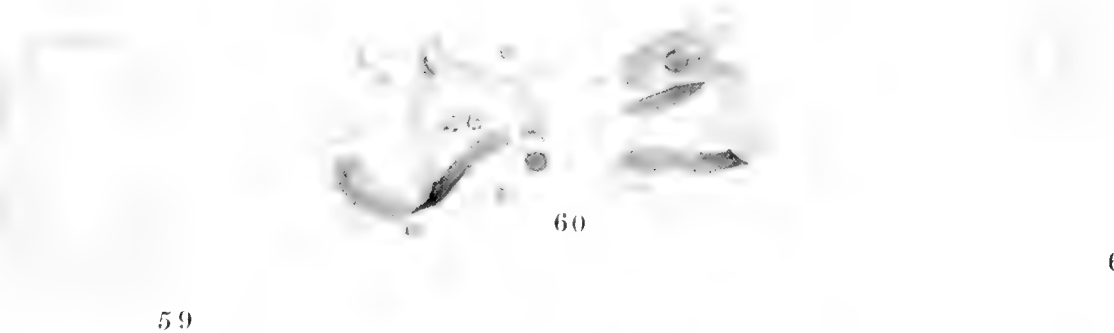
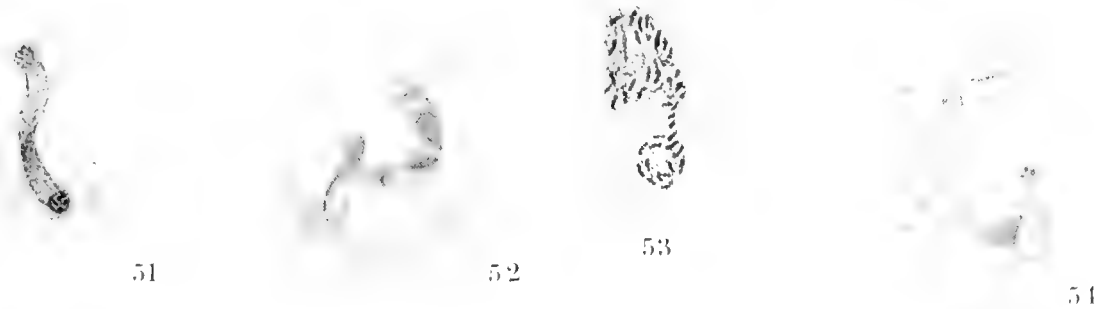
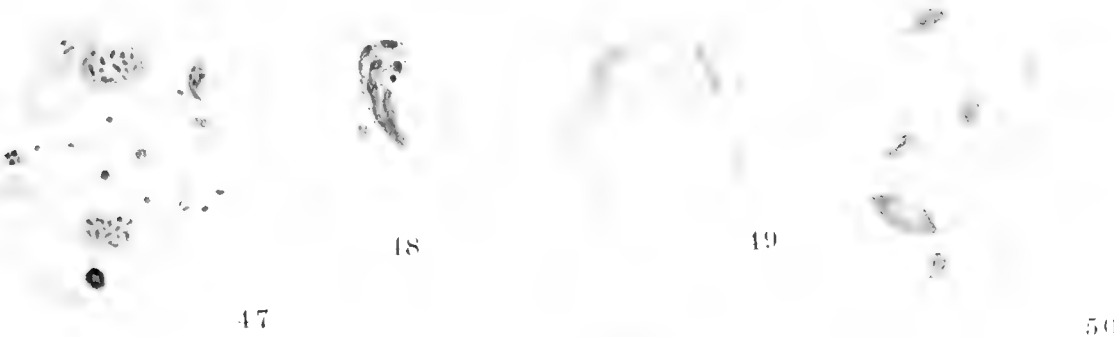
53 A portion of the nucleus pictured in figure 65, showing elongated chromatin granules. $\times 787$.

54 Early division stage drawn from a total preparation to show elongated nucleus and three vesicular bodies. $\times 415$.

56, 57, 58, 59, 61 A series of division stages drawn from the living animal.

60 Total preparation of a late division stage to show the elongated nuclei and two vesicular bodies. $\times 415$.

62, 63, 64, 65 Serial sections of a dividing cell, showing comma-shaped and thread-like bodies scattered throughout the endoplasm. A section of the nucleus is shown in figures 64 and 65. $\times 415$.



62
JEM del

THE HEART AND ARTERIES OF POLYODON

C. H. DANFORTH

From the Department of Comparative Anatomy of Harvard Medical School

NINETEEN FIGURES

The following account of the arterial system of the ganoid Polyodon was begun in the Anatomical Department of Washington University and completed in the Department of Comparative Anatomy at Harvard Medical School. The writer gratefully acknowledges his indebtedness to the staffs in both places. The work is approached entirely from a morphological viewpoint and only the gross anatomical relationships are considered. The material used has been chiefly fish of about a meter in length which were secured in the vicinity of St. Louis. These were injected with gelatin and starch masses, the latter proving quite satisfactory for present purposes. The results have been checked by a study of serial sections of a 74 mm. specimen which has already been briefly described by the writer (Danforth, '11).

Allis's ('11) paper on the pseudobranchial and carotid arteries of Polyodon did not appear until the present work had been finished except for the drawings. Since that paper only partially covers the field attempted here and moreover some comparison of our results seems desirable the paragraphs on the pseudobranchial and carotid vessels are retained without very much condensation.

PERICARDIUM AND HEART

The pericardium has the usual conical or rounded form with the base directed caudally against the septum transversum. A dorsal and two lateral faces are vaguely indicated. The lining is a uniform serous membrane with no macroscopic openings except that of the pericardio-peritoneal canal. This is a rather

large passage which opens from the pericardium into the coelom, the posterior mouth being in the usual position ventral to the oesophagus and dorsal to the liver. The pericardial mouth opens dorsally between the entrance of the right and left ducts of Cuvier. In a specimen where the greatest lateral diameter of the pericardial cavity was 36 mm., the smallest diameter of the pericardio-peritoneal canal was 5 mm. The canal itself was about 15 mm. long.

The heart is attached to the pericardial wall anteriorly by the truncus arteriosus and posteriorly by the union of the great veins with the sinus venosus, by two cords consisting each of a coronary vein and a posterior coronary artery, and by fine strands running from the septum transversum to lymphoid masses on the ventricle. The attachment of the sinus to the pericardial wall is somewhat in the form of an upright H, of which the great veins form the limbs. In the dorsal notch of the H is the pericardial opening of the pericardio-peritoneal canal and through the ventral notch pass the coronary vessels. These are in two free strands each consisting of an artery and a vein. Usually the left vein and right artery are large and the right vein and left artery small. The veins were not studied in detail; the arteries will be discussed further on.

The sinus venosus is very asymmetrical. On the left its antero-posterior diameter is short so that the veins almost enter the auricle, but on the right, lateral to the mouth of the veins, there is a saccular dilation equal in capacity to about one-third of the auricle. Posteriorly there are a few internal trabeculae or reinforcing strands, but these are not much developed. The arterial blood supply, is in part at least, by small twigs of coronary origin coming in from the septum transversum.

Externally the auricle appears nearly symmetrical but slightly inclined to the left. It has a smooth surface and is somewhat crenulated around the margin. In sagittal section it is in the form of a right triangle, high behind and low and thin in front. Within there is a strong development of trabeculae, mostly flattened and forked, all around the edge except for a short distance on the left, lateral to the auriculo-ventricular opening.

Thus there are no trabeculae crossing the region opposite the opening, but a crescent of them radiates around it, their contraction doubtless focusing the blood on this point. The weakest place in the wall, therefore, is opposite the mouth. The sino-auricular valve is a pair of folds on the left placed oblique to the sagittal plane with the ventral end of the slit between them more medial than the dorsal. The slit makes an angle of about 45 degrees with the perpendicular. The nearly round auriculo-ventricular opening, is placed lateral, ventral, and anterior to the opening from the sinus. It is guarded by the auriculo-ventricular valve shown in fig. 14 *B*.¹

The ventricular part of the heart is almost completely surrounded and concealed by large lobes of lymphoid tissue appended to its outer wall and richly supplied from the coronary artery. Otherwise it presents no features calling for special mention.

The conus arteriosus of *Polyodon* is well developed. The number of valves, however, is relatively few as compared with some other ganoids. Figs. 1 and 2 each represent a conus that has been cut along the mid-ventral line and opened to show the cusps of the valves. In fig. 11 a portion of the lateral wall has been removed exposing the valves within. It will be seen from these figures that in this species the conus is a variable structure, at least in regard to the number of valves. Perhaps the most common form has three valves of four cusps each, the cusps being arranged in longitudinal rows as is usual among elasmobranchs and ganoids. Between the first and second valve there is a considerable space. In some individuals this space is occupied by another valve which may be either rudimentary (fig. 11), or well developed (fig. 2). Although there are typically four more or less equal cusps to each valve their number and form vary and all stages from the merest rudiment to well developed cusps can be found. In some cases, as in the second tier shown in figure 1, multiplicity seems to result from a division of one of the cusps. In agreement with most other forms the first (most anterior) valve, supposed to correspond with the single valve

¹The figures in this paper were made by Mr. Wm. T. Oliver from the original drawings by the writer.

ABBREVIATIONS

- a.bi.*, terminal bifurcation of afferent branchial arteries
a.br.a. (1, 2, 3, 4), afferent branchial artery
a.br.e. (1, 2, 3, 4), efferent branchial artery
a.cc., common carotid
a.ce., external carotid
a.ci., internal carotid
a.co-ca., arteria coraco-cardiaca
a.coe., coeliac artery
a.com., branch of external carotid to region of pseudobranch
a.co-me, coeliaco-mesenteric artery
a.cn., artery of heart
a.cor., coronary artery
a.dor., dorsal aorta
a.eps., efferent pseudobranchial artery
a.fa., facial artery
a.fg., artery to the F-shaped groove of Bridge
a.fil.a., afferent filamentar artery
a.fil.e., efferent filamentar artery
a.fil.s., basal branch of afferent filamentar artery
a.fil.tr., transverse filamentar artery
a.fil.x., network of filamentar branches
a.hb. (2, 3, 4), hypobranchial artery
a.hb.y., a terminal branch of median hypobranchial artery
a.he.a., anterior hepatic artery
a.he.p., posterior hepatic artery
a.hy., afferent hyoidean artery
a.hyo., hyoöpercular artery
a.an., innominate artery
a.lhb., *lhb.'*, *lhb. ''*, *lhb. '''*, *lhb. ''''* possible rudiments of lateral hypobranchial arteries
a.md., afferent mandibular artery
a.mhb., median hypobranchial from second recurrent arteries
a.mhb.', median hypobranchial from fourth recurrent arteries
a.nu., nutrient artery of gill
a.om., arteria ophthalmica magna
a.on., orbitonasal artery
a.op., ophthalmic branch of orbitonasal artery
a.pa., parietal arteries
a.ph., pharyngeal branch of second efferent artery
a.rc., recurrent branch of afferent branchial artery
a.re., artery to kidney
a.rec., rectal artery
a.ret., arteria retinalis
a.sc., subclavian artery
a.sp., subpericardial branch of coronary artery
a.spl., splanchnic artery
a.sthy., artery to the m. sternohyoideus
a.thd., arteria thoracico-dorsalis (?)
a.thv., arteria thoracico-ventralis (?)
au., auricle
bd., bile duct
br., branchiostegal ray
c.br., branchial cartilage
c.cer. (1, 2, 3, 4), ceratobranchial cartilage
c.ch., ceratohya cartilage
c.co. (1,2,3), copulae
c.ep. (1,2,3,4), epibranchial cartilage
c.fi., filamentar cartilage
c.hyb. (1,2,3,4), hypobranchial cartilage
c.hyh., hypohyal cartilage
c.hyo., hyomandibular cartilage
c.i.h., interhyal cartilage
c.mc., Meckel's cartilage
c.pbr. (*pbr.'* *pbr. ''*), pharyngobranchial cartilages
c.sym., symplectic cartilage
ca., caeca
co., coelom
con., conus arteriosus
div., spiracular diverticulum
gb., gall bladder
gr., gill rakers
hb.a., anterior hemibranch
hb.p., posterior hemibranch
l., liver
lig., longitudinal ligament of dorsal aorta

lym., lymphatic vessel
m.adm., m. adductor mandibularis
m.adh., m. adductor hyomandibularis
m.bmd.m.bmd., 'origin and insertion
 of m. branchiomandibularis
m.lev. (1,2,3,4), m. levator arcuus
 branchialis
m.obv. (1,2,3,4), m. obliquus ventralis
m.pr., m. protractor mandibularis
m.sthy., tendon of sternohyoideus mus-
 cle
no., notochord
oc., oral cavity
oper., operculum
os.den., dentary bone
os.mx., maxillary bone
os.pa., parasphenoid bone
os.ps., post scapula

pc., pericardial cavity
rec., rectum
rsp., respiratory folds
sb., swim bladder
si., small intestine
spi., spiracle
spl., spleen
st., stomach
sv., sinus venosus
thy., thyroid gland
v.dc., duct of Cuvier
v.hp., hepatic vein
v.j., jugular vein
v.ji., inferior jugular
v.p., portal vein
c.pc., post cardinal vein
ven., ventricle

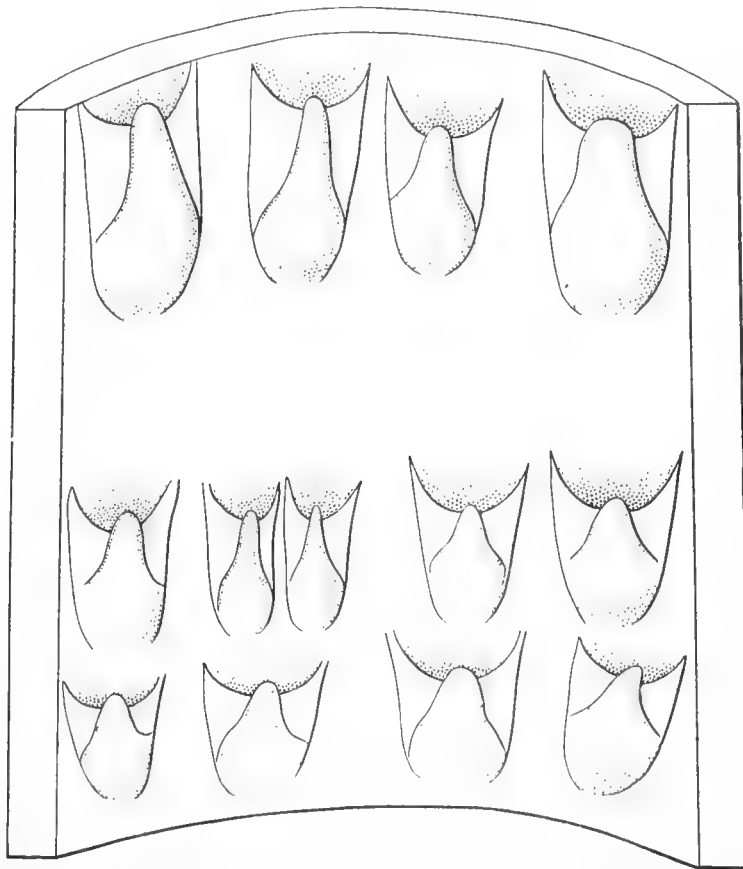


Fig. 1 A conus arteriosus cut along the midventral line and spread open. In this specimen there are but three valves, one of which has an extra cusp.

of teleosts, is usually the one best developed. The cusps themselves are of the characteristic type described by Stöhr ('76), having a thick middle portion and thin lateral attachments that are more or less fenestrated. There are also numerous strands on the inner side of the flaps. Stöhr ('76) and Boas ('80) have published accounts of conus forms in a number of the ganoids, but neither of them includes *Polyodon* in his descriptions. These authors point out that a 'beautiful transi-

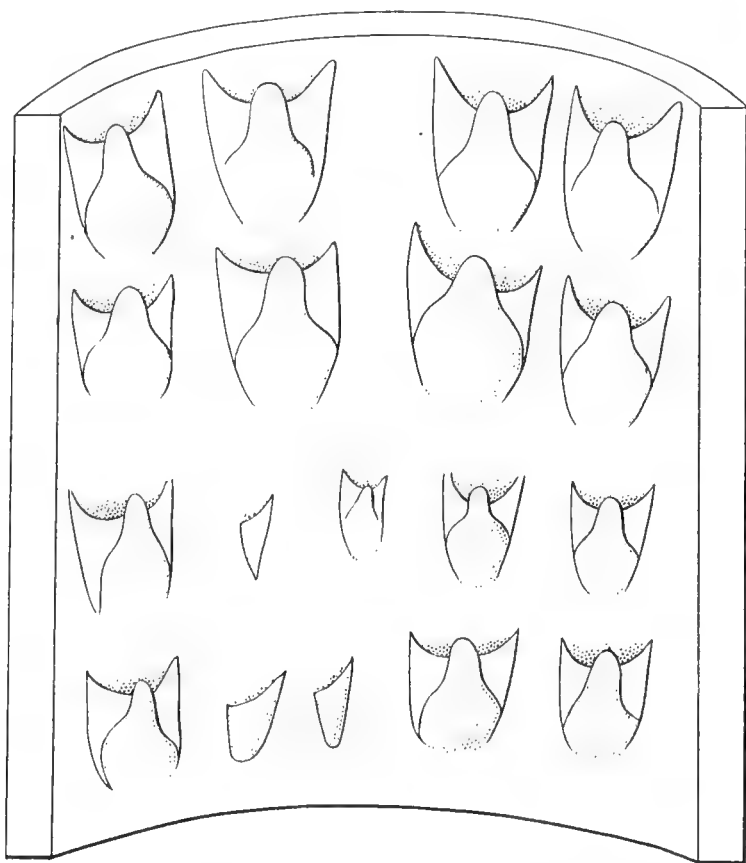


Fig. 2 A conus with four well developed valves

tion' from the ganoid to the teleostean type of conus is found in the hearts of *Amia* on the side of the ganoids and of *Butyrinus* among the teleosts. The former has three valves of four cusps each. Of these cusps two are reduced in size in each valve. *Butyrinus* has two valves, the anterior with two cusps, the posterior with four as in *Amia*. Senior ('07) has recently described the conus of *Tarpon* which contains two valves of but two cusps each. With these exceptions almost all teleosts have a single

valve of two cusps. If it were desirable to show a still closer series of gradations, the variable conus of *Polyodon* might be placed before that of *Amia* in a series leading back through such forms as *Lepidosteus* and *Polypterus* to the complicated structure found among the Dipnoi. Such a finely graded series, however suggestive it might be, would probably not correspond to any line of descent. The morphological value of the exact number and arrangement of these valves cannot be very great, especially where there is a tendency to multiplicity.

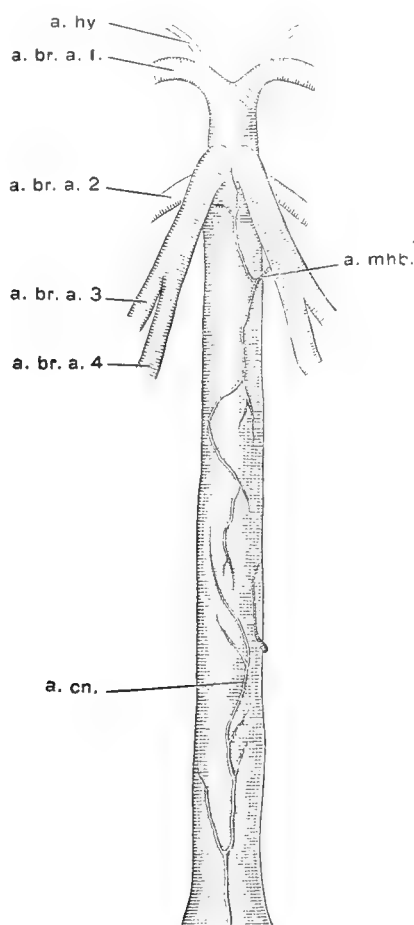


Fig. 3 Dorsal aspect of the ventral aorta

VENTRAL AORTA AND AFFERENT BRANCHIAL ARTERIES

The ventral aorta (fig. 3) is greatly elongated. In a fish of 10 decimeters it is fully one-tenth of the entire length of the specimen. All of its branches arise near together at the anterior

end. They are disposed in three pairs, morphologically comparable to similar vessels in elasmobranchs. The most anterior, resulting from a terminal bifurcation of the aorta, is a short trunk on either side, which presently divides to form afferent hyoidean (*ahy.*) and first branchial (*a.br.a. 1*) arteries. Next behind this comes a paired vessel which arises from the dorsal aspect of the aorta and supplies arteries (*a.br.a. 3*, *a.br.a. 4*) to the third and fourth gills. Finally the afferent artery (*a.br.a. 2*) to the second gill, which is the only one to come directly from the aorta, is in point of origin the most posterior of all. This is due, of course, to the displacement headwards of the common trunk of supply to the third and fourth gills.

The afferent hyoidean (fig. 4, *a.hy.*) and first branchial (*a.br.a.1*) arteries immediately pass ventral to the tendon of the M. sternohyoideus (*m.sthy.*), while all the other afferent arteries are dorsal to it. The former, running close beneath the skin, follows the hyoid arch for a considerable distance. Anteriorly it lies just medial to and parallel with the A. hyoidea (*a.mdl.*), the vessels and the cartilage suggesting the arrangement of parts in a gill. Further back it does not follow the arch so closely and runs more nearly parallel with the median line. This vessel seems clearly to correspond to the afferent hyoidean artery of other ganoids and selachians. With teleosts, although sometimes present in young (e.g., *Ameiurus*, *Salmo*), it appears typically to be absent in the adult. In *Polyodon* I have frequently been unable to find it, so its absence may here be a more or less common anomaly—an anomaly which might perhaps be expected on physiological grounds inasmuch as this artery can distribute only venous blood to the tissues.

The first afferent branchial artery, after having passed under the tendon of the sternohyoideus, turns obliquely outward and backward to enter its gill along the postero-ventral border of the m. obliquus ventralis I (*m.obv.1*). The second enters its gill in exactly the same way except that it goes dorsal instead of ventral to the tendon (fig. 4).

The trunk (*a.an.*) on either side which supplies the third and fourth gills runs back near the median line and dorsal to the

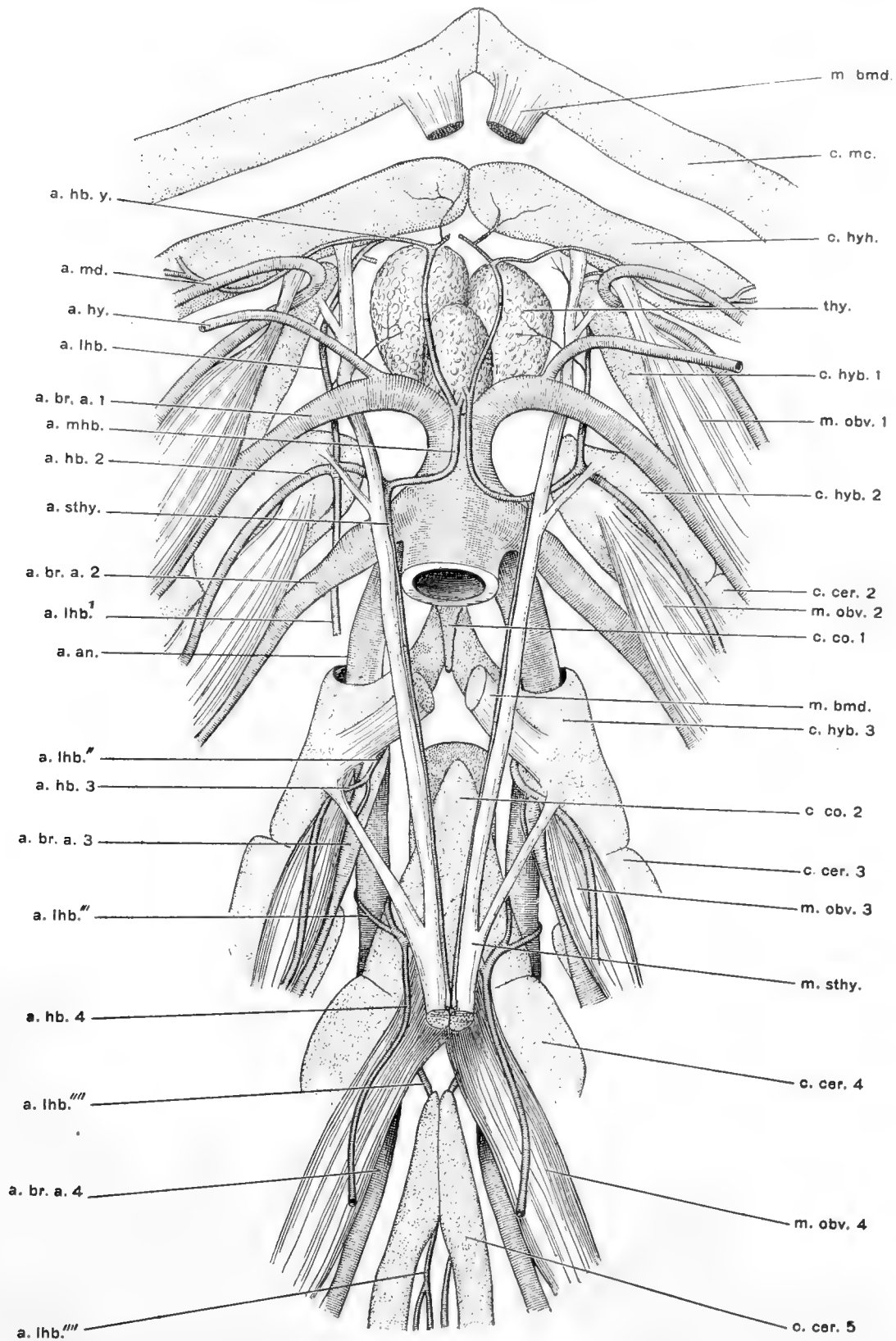


Fig. 4 The principal structures of the hypobranchial region seen from below. The anterior cartilage elements are displaced somewhat laterally and portions of the copulae omitted.

aorta. At the level of the third hypo-branchial cartilage it divides into the two afferent arteries. One (*a.br.a. 3*) enters the third gill in the same way as those described above, while the other (*a.br.a. 4*) reaches the fourth in what at first sight appears a very unusual manner. It ascends in an oblique groove on the lateral face of the second copula (fig. 5) to gain the floor of the mouth where it is separated from the oral cavity only by the mucosa and the slightest amount of subjacent tissue (fig. 6, *A*). It crosses dorsally the anterior end of the ventral cartilage of the fourth branchial arch and then turns down in a groove on the medial and posterior aspect of that cartilage to enter the gill along the *m. obliquus ventralis IV*, thus coming into corre-

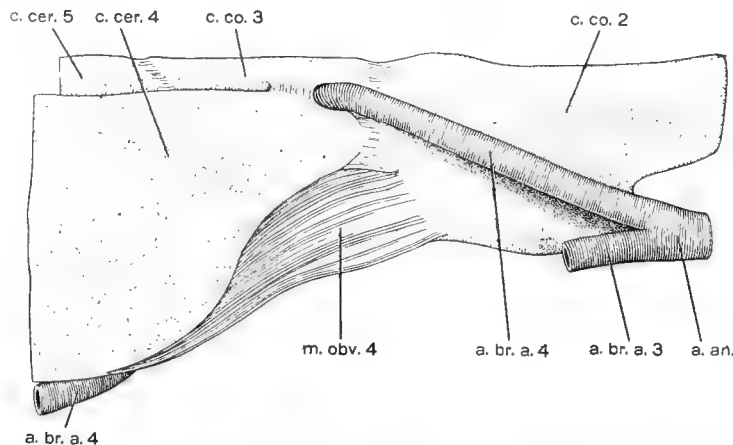


Fig. 5 The origin and proximal relations of the fourth afferent branchial artery.

spondence with all the more anterior afferent arteries. Caudad to the groove for the artery there is a ligament binding the branchial cartilage to the second copula, and this is interpreted by Bridge ('79) and Van Wijhe ('82) as a second articulation. Such an interpretation seems justifiable, and, if it be correct, brings *Polyodon* into accord with *Amia* and *Acipenser*, in so far as this point is concerned. In the third and fourth arches of *Amia* the hypobranchial articulates with the copula near the floor of the mouth and with its ventral keel at a deeper level (fig. 6, *B*). The artery passes through the enclosure thus formed. With *Polyodon* the same condition exists in the third arch, but in the fourth, where the hypobranchial has disappeared or lost

its individuality, the ventral articulation is displaced forward and upward to the level of a dorsal articulation, and the original dorsal (if it be such) has become posterior in position and is nearly obliterated. In this readjustment the artery (*a.br.a.* 4) has retained its original relative position and is in consequence brought up to the floor of the mouth (fig. 6, A).

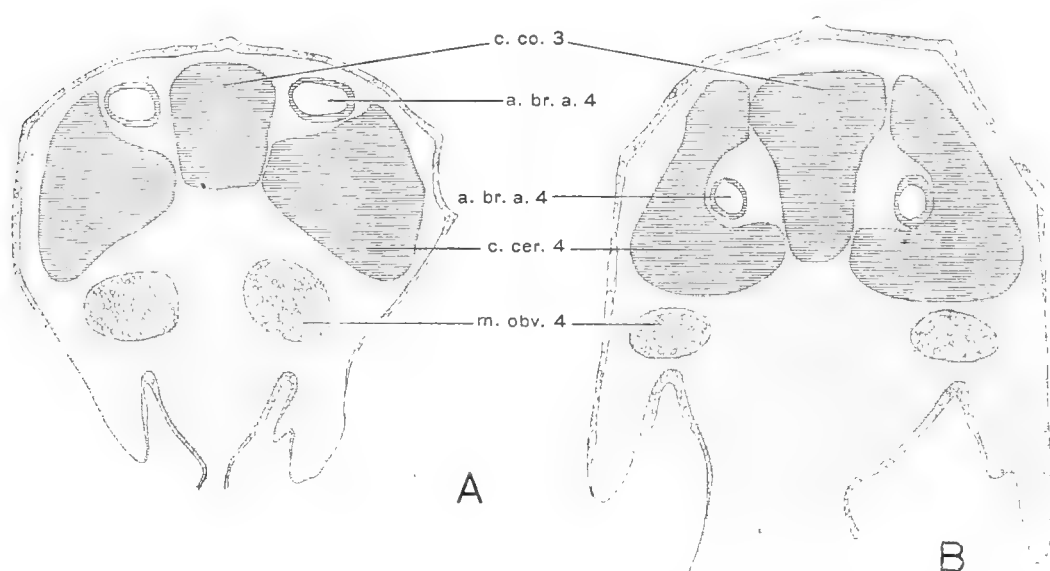


Fig. 6 A, section through a portion of the hypobranchial region of *Polyodon* (74 mm. specimen); B, a similar section of *Amia* at the same level (Harvard Embryological Collection, No. 273, sections 270-285).

THE BRANCHIAL CIRCULATION

On entering their respective gills the afferent vessels (*a.br.a.*) run a considerable distance without giving rise to any filamentar arteries. At a point well towards the middle of the ceratobranchial region each gives off a recurrent vessel (*a.rc.*, fig.7) which bears all the filamentar arteries medial to this point. Dorsally all the afferent vessels except the fourth bifurcate near the end of the gill, sending a division to the two hemibranchs as they diverge around the insertion of the *m. levator arcus branchialis* (*a.bi.*). Allis (loc. cit., p. 259) discusses these vessels briefly, but does not mention their terminal bifurcation. He finds the recurrent branches in larvae (130 mm. to 170 mm.), but in view of drawings in his possession thinks they may be

absent from the first arch in the adult. The present writer finds them occurring invariably in all the arches.

The efferent arteries, with the partial exception of the fourth, to be described presently, parallel very nearly the afferent vessels as indicated in figure 7. They arise ventrally by two long branches, one from each hemibranch, which unite at about the same level as that at which the recurrent afferent artery is given off. This corresponds very well with what Silvester ('04) found to be the

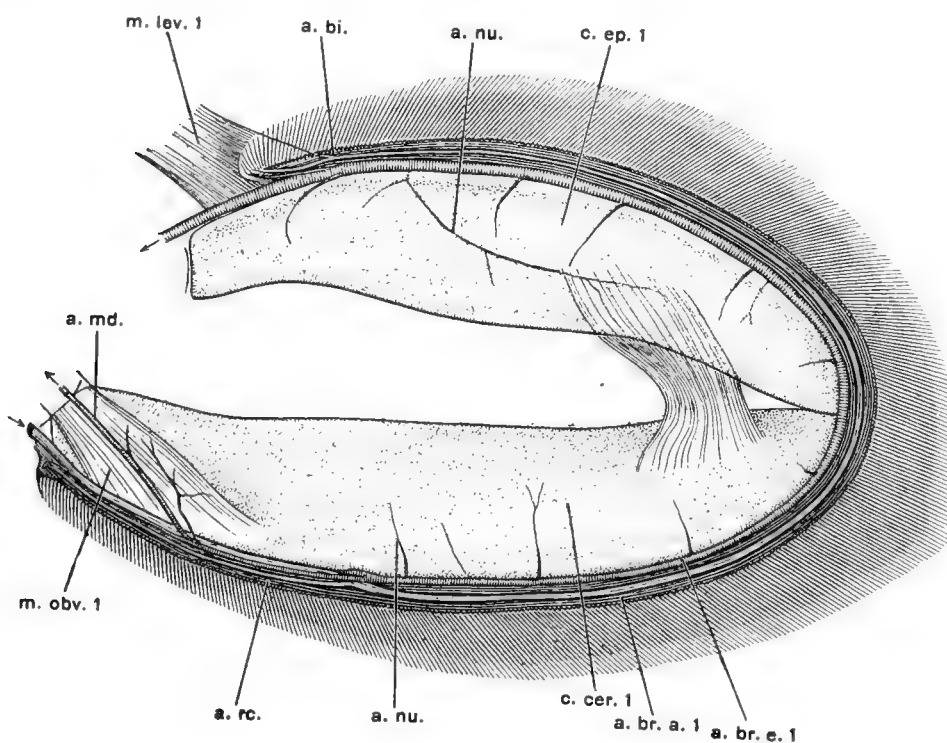


Fig. 7 Anterior aspect of the first gill after the removal of some of the superficial tissues.

condition in a large number of teleosts. Dorsally the efferent vessels all send off small branches to accompany the terminal bifurcations of the afferent arteries. Throughout the whole gill the efferent filamentar arteries tend to unite in tree-like groups (cf. fig. 9), a single stem often draining several filaments. Morphologically the dorsal and ventral branches may be simply enlarged stems of this sort that have developed with the increased size of the gill. If such be the case they probably do not indicate an incomplete fusion of a pair of efferent vessels such as occurs

in each holobranch of sharks, as Allis seems to suppose. As shown in the figure, numerous nutrient arteries (*a.nu.*) are given off all along the gill. Largest and most important of these are the hypobranchials discussed below, which arise from the anterior ventral divisions of the efferent arteries.

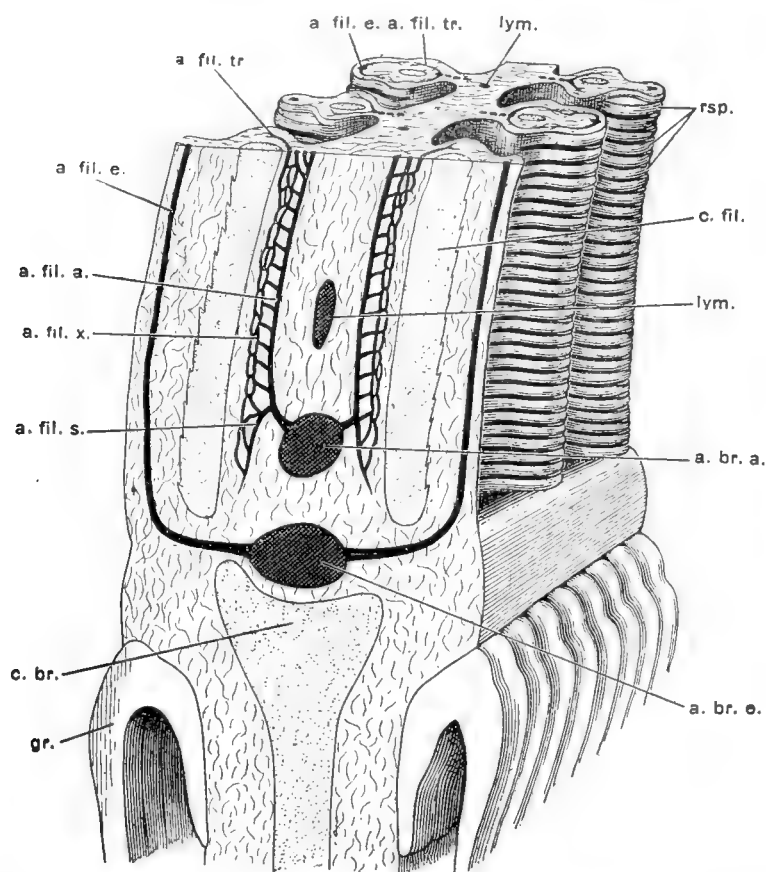


Fig. 8 Stereogram of a region near the middle of a gill. The basal parts of two and one-half gill-filaments are shown on each side.

The arrangement of vessels near the middle of a gill is shown semidiagrammatically in figure 8. This arrangement is described by Allen ('07, p. 106). The efferent artery (*a.br.e*) lies deepest and next above it is the afferent artery (*a.br.a*). The third vessel (*lym.*) still higher is the branchial vein or lymphatic of Allen's account. The afferent filamentary artery at its origin immediately gives off a short spur (*a.fil.s.*) to supply the region lateral to the main vessel and below its own point of origin.

The main stem (*a.fil.a.*) ascends to the top of the filament, giving off throughout its whole length a series of short cross pieces which reunite in an irregular network (*a.fil.x.*) from which the 'afferent transverse filamentar arteries' (*a.fil.tr.*) take origin. Each of these supplies several of the flap-like folds of respiratory epithelium (*rsp.*) on either side of the filamentar cartilage (*c.fil.*). From the ultimate capillaries the blood is returned directly to the efferent filamentar artery (*a.fil.e.*) which descends on the other side of the cartilage to pass through a notch in its base.

It is stated above that the efferent branchial artery of the fourth gill varies from the corresponding vessels anterior to it. This divergence is correlated with other modifications in the region. The fourth gill, unlike the others, is not a complete holobranch. Its anterior hemibranch (*fig. 9, hb.a.*) is entire but the posterior, (*hb.p.*) although constantly present, extends only slightly into the epibranchial region where a fusion has taken place, obliterating the dorsal half of the cleft. The ventral half of the cleft, however, remains open. This simple but unusual condition has proved misleading to several writers. Van Wijhe (*op. cit.*) says that each of the four first gill-arches bears on its outer side a whole gill. Jordan ('99) diagnosing the family Polyodontidae, says 'gills $4\frac{1}{2}$ '. Finally Allen twice states (*l.c.*, p. 106, p. 108) that, "the fourth or last branchial arch has but one row of filaments, and is therefore a hemibranch." Strictly speaking all of these descriptions are incorrect. Apparently Van Wijhe failed to notice the absence of the dorsal half of the fourth hemibranch, while Allen overlooked the presence of the ventral half. Jordan's statement is probably based on the cartilaginous arches and not on the gills themselves. A considerable number of specimens examined by the present writer proved very uniform in this particular except that in a young one, 74 mm. long, the filaments were found to be rudimentary in the epibranchial region of all the gills. In its lower half the fourth gill is like those in front of it and its efferent artery gives off the characteristic hypobranchial vessel in the usual manner.

Near the point where it rounds the articulation between the epi- and cerato-branchial cartilages, the fourth efferent artery

gives off a large posteriorly directed branch (figs. 9, 11 *a.cor.*), which for the present may be termed the coronary artery. It has a wider range of origin (cf. figs. 10 and 11) than is implied by Allis's account, but always leaves the gill in the epibranchial region, and this, in connection with the fact that there is already a full complement of recurrent arteries below, precluded the

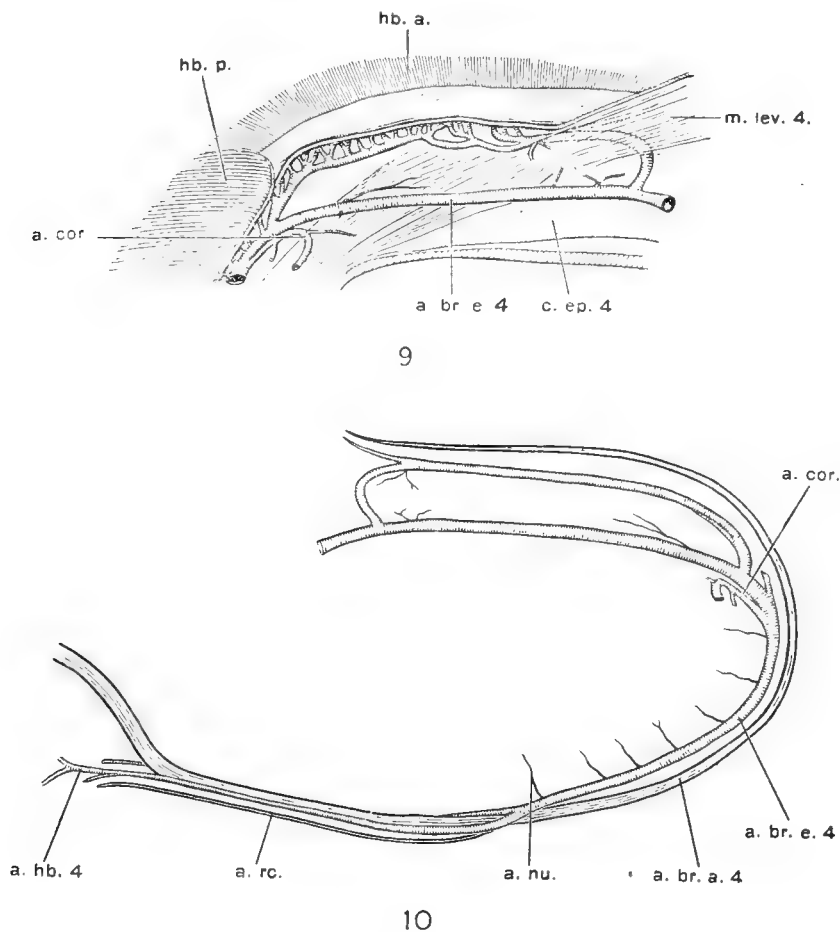


Fig. 9 Posterior view of dorsal part of fourth gill

Fig. 10 The arteries of the fourth gill

possibility of its belonging to the hypobranchial system. Passing over the dorsal end of the fifth gill cleft it comes into a position lateral to the oesophagus from which point its course will be traced in a subsequent paragraph.

Near the origin of the 'coronary artery' and in the region where the posterior hemibranch is lacking (fig. 9), the efferent vessel separates into two parts. The main division (*a.br.e. 4*), which

gives rise only to nutrient branches, passes over the posterior face of the muscle connecting the cerato- and epi-branchial cartilages and across the epibranchial directly to the pharynx. The other part (figs. 9, 10), which gives rise to both filamentar and nutrient branches, follows the dorsal edge of the cartilage and supplies the filaments of the anterior hemibranch. It goes anterior to the attachment of the m. levator arcus branchialis and reunites with the vessel from which it arose. Allis infers from Allen's drawings that the loop thus formed is interrupted medially in the adult, but such is not always the case, for in all the specimens which were examined by the writer the loop was found to be complete as shown in the figures.

Although the filaments of each of the first three posterior hemibranchs are practically in a continuous line above and below with those of the next succeeding anterior hemibranchs, the eby completely surrounding the clefts with filaments, I have been unable by gross methods to find any connection at this point between the arteries of the successive gills. Allis, however, finds an indirect connection between the arteries of the third and fourth gills.

THE HYPOBRANCHIAL ARTERIES

In all groups of fishes a system of hypobranchial arteries is of constant occurrence, but the details of arrangement are rather variable. These arteries are derived from anastomoses between recurrent vessels which arise from the efferent arteries within the gills. Frequently secondary longitudinal trunks are formed which from their position T. J. Parker ('84, '86) has designated the lateral and median hypobranchials. Their transverse connections he calls commissural arteries. That author, it may be observed, regarded the whole system as derived from the subclavian artery because in the sharks, with which he worked, there is a connection between the two. The connection with the gill arteries he thought to be secondary. This view, however, has received little subsequent support and is not now generally maintained. G. H. Parker and F. K. Davis ('99)

corrected and elaborated the original account and extended their observations to one of the ganoids, *Amia*. Parker ('00), Silvester ('04) and others have studied these vessels in teleosts. In *Polyodon* I find a considerable latitude of variation in minor points even on opposite sides of the same fish, but no asymmetry of constant occurrence, such as characterizes some of the teleosts, has been noticed. In figure 4 there is a slightly schematic representation of the whole system in a specimen where the parts attained a very full development.

The recurrent artery from the first gill (*a.md.*), generally designated as *A. hyoidea* or afferent pseudobranchial artery, is apparently constant in occurrence and position. It does not anastomose with the other hypobranchial arteries and in this region gives off only very small branches or none whatever. From its origin (fig. 7) it crosses the *m. obliquus ventralis I* diagonally and loops around its tendon, passing first dorsal, then medial and finally ventral to it as shown in figure 4. On rounding the tendon it gains the postero-medial aspect on the hypohyal where, however, there is neither foramen nor groove. Allis ('11) finds branches here to the local musculature. It crosses the lateral aspect of the ceratohyal (*c.ch.*) in a diagonal furrow (fig. 16) which traverses nearly the whole length of the proximal cartilaginous portion of that element. Turning dorsally it passes in front of the interhyal to the lower edge of the symplectic where it again enters a shallow groove in the cartilage. Here it frequently gives rise to a vessel (fig. 16) that runs back under the end of the hyomandibular. Allis (l.c., p. 260) is convinced that this branch is homologous with similar vessels in *Amia* and *Salmo* and that it represents in all these the "remnant of a commissure that in younger larvae undoubtedly connected the hyoidean and mandibular aortic arches." It is absent in many of the adult *Polyodons* studied. Leaving the dorsal aspect of the symplectic the afferent pseudobranchial artery runs forward in a course ventral to the protractor hyomandibularis muscle, but separated from the oral mucosa by a thick layer of fatty tissue. Anteriorly it makes an *S*-shaped bend (fig. 15) and enters the pseudobranch, where it usually breaks up into several

divisions, from which the ultimate filamentar arteries (about twenty) arise.

This vessel and the afferent hyoidean artery referred to above apparently correspond to the similar arteries in *Amia* and the young trout. These vessels Allis ('00) and Maurer ('88) believe on embryological grounds to be the true afferent arteries of the first and second arches, and this would seem to be the implication of Greil ('03) in reference to elasmobranchs. But Wright ('85) working with *Lepidosteus*, and Silvester ('04) with teleosts are inclined to other interpretations. It can hardly be profitable to discuss this question in connection with *Polyodon* until something of its embryology is known.

The hypobranchial arteries posterior to the hyoidean are more variable and tend to become asymmetrical. The recurrent vessel (fig. 4, *a.hb.2*) from the second gill, when fully developed, on entering the hypobranchial region passes behind and dorsal to a small accessory tendon from the m. sternohyoideus to the second branchial cartilage. Here it gives off an anterior branch (*a.lhb.*) which passes dorsal to all other arteries and tendons to supply the lateral lobe of the thyreoid and the articular surfaces of the anterior basibranchials. At about the same place it also gives rise to a posterior branch (*a.lhb'.*) which runs back dorsal to the second afferent artery to reach the ventral aspect of the third basibranchial cartilage. The recurrent vessel itself, turning slightly backward and inward, presently gives off a third branch (*a.sthy.*). This is to the sternohyoideus (*m.sthy.*), the tendon of which it follows along the dorso-medial side. It generally passes laterad of the m. branchiomandibularis (*m.bmd.*), but may go mesad of it. In one specimen, unfortunately injured, the vessel seemed to encircle the muscle. The vessels of the two sides meet behind on the belly of the sternohyoideus but remain more or less distinct. After giving off these three branches the main trunk of the recurrent artery reaches the median line ventral to the aorta, where it meets a corresponding artery (if present) from the other side. Frequently the two vessels (*a.mhb.*), usually of unequal size, run forward side by side without uniting. They supply the median lobe, and possi-

bly more, of the thyreoid and terminate as the nutrient arteries of the branchiomandibularis (*m. bmd.*) which they reach by passing between its two tendons of insertion to gain the ventral side of the muscle. But while still dorsal to the branchiomandibularis several minor branches are given off. One of these (*a.hb.y.*) is very constant in its occurrence. It arises near the median line and runs laterad beneath and close to the insertion of both the sternohyoideus and transversalis I. It here comes into contact with the hyoidean artery, twice crossing its dorsal surface, but apparently never anastomoses with it. Its final distribution is over the hypobranchial cartilage and the surrounding connective tissue.

In these vessels what may represent lateral, medial and commissural elements can, perhaps, all be recognized, but this is a point which should not be pressed too strongly, for the vessels are all variable and it is doubtful if they have much significance for comparative purposes. The two lateral branches could together be interpreted as constituting a lateral hypobranchial artery, and the fact that the anterior division sends a twig to the thyreoid is in agreement with that vessel in the elasmobranchs as described by Ferguson ('11). Whether or not the posterior branch ever effects a communication with the arteries from the third or fourth gills I cannot say. Apparently it usually does not, although in some cases the terminal twigs come very close to one another. The portion of the recurrent vessel between the 'lateral hypobranchial' and the middle line is in the correct position for a commissural artery and the rest of the vessel (*a.mhb.*) is the median hypobranchial. It is in accordance with teleostean conditions, according to Silvester ('04) and Gudernatsch ('11), for the median hypobranchial to supply the thyreoid. The supply of the median part of the thyreoid, when this vessel is lacking, has not been fully determined, but apparently the gland then draws exclusively on the lateral hypobranchials. The median hypobranchial is not produced backward beneath the aorta as it is in almost every fish thus far described, but all the derivatives of the second afferent artery are confined to the region anterior to the third gill.

From the third gill there may or may not be a recurrent vessel. When present it is comparable to the others in its general relations but has a rather limited distribution.

The fourth efferent artery, so far as my experience goes, invariably gives off a recurrent vessel which has median and lateral branches. From the former are derived the anterior vasa of the ventral aorta (fig. 3 *a.mhb.*') and other small twigs. The lateral branches, possibly to be considered collectively as posterior remnants of the lateral hypobranchial, are anterior and posterior as regards their direction. The former (*lhb.*''') is small and may anastomose anteriorly with a similar vessel from the third gill when there is one present. The main trunk of the posterior branch (*lhb.*'''), which is larger and more diffuse in its distribution, gains the median aspect of the fifth ceratobranchial and runs back for a considerable distance. Its terminal twigs reach those of the vessels from behind the heart (fig. 12) and may even anastomose with them, but any connection here is probably only secondary. In distribution, but not in origin, it suggests the dorsal median hypobranchial artery of Silvester's account.

The restricted distribution of the hypobranchial arteries of *Polyodon* is probably to be correlated with the fact that the hypobranchial region as a whole is greatly reduced and pushed far forward so that the direct connection between this and the pectoral region is unusually slender, consisting for a considerable distance of little but cartilage and tendons. The great variability of the minor vessels on the other hand is a characteristic shared by fishes in general. But that there should be no coronary artery in connection with this system is interesting and apparently unique. Allen (op. cit., pl. 3, fig. 5) shows a vessel arising in the second gill and indicated as 'coronary artery.' This figure was made to show lymphatics, however, and is almost certainly incorrect so far as the artery is concerned. The vessel in question is either the commissural or the artery of supply to the sternohyoideus; and Allen himself states in the text that the coronary artery "comes from the fourth right efferent branchial artery and approaches the heart from the rear." This

is true, but the statement fails to make clear the important fact that the coronary does not come from the hypobranchial end of the efferent artery as in other fishes. Allis does not discuss the hypobranchial arteries.

THE CORONARY ARTERY

The coronary arteries as usually described for fishes are designated as anterior and posterior. The former arise from hypobranchial derivatives of the efferent branchial vessels and reach the heart by passing along the conus arteriosus. The latter, found in skates, arise from the coracoid branch of the subclavian artery and reach the heart from behind. Depending on their relation to the conus, anterior coronary arteries are recognized as dorsal or ventral. In *Polyodon* anterior coronary arteries are all entirely lacking and, further, the arteries of supply which reach the heart by way of the septum transversum do not correspond in other respects to posterior coronaries.

The vessel which for convenience may be called the coronary artery (figs. 11 and 12 *a. cor.*), although it represents much more than is usually covered by that term, arises as above described from the fourth efferent branchial artery which it leaves in the epibranchial region. It is somewhat variable in its point of origin (figs. 9, 10 and 11, *a. cor.*) and is not symmetrical with the corresponding vessel of the opposite side, one or the other always being much the larger. Usually it is the right vessel that is best developed. In the region of the oesophagus it gives off a few small branches and then descends in a somewhat spiral course to the base of the pericardium. Its relation in this region to the anterior cardinal and ductus Cuvierii (fig. 11, *v. dc.*) is variable. It lies for the most part anterior and somewhat medial to the subclavian artery (fig. 12, *a. sc.*). Dorsal to the pericardium there arises a long branch (*a. co.-ca.*) that runs forward between the fifth ceratobranchial and median hypopharyngeal cartilages to supply the dorsal parts of the mm. pharyngoclaviculares (fig. 12). This vessel occupies the position of a part of the *a. coraco-cardiaca* of *Scyllium* (Hyrtl '58, Carazzi '04),

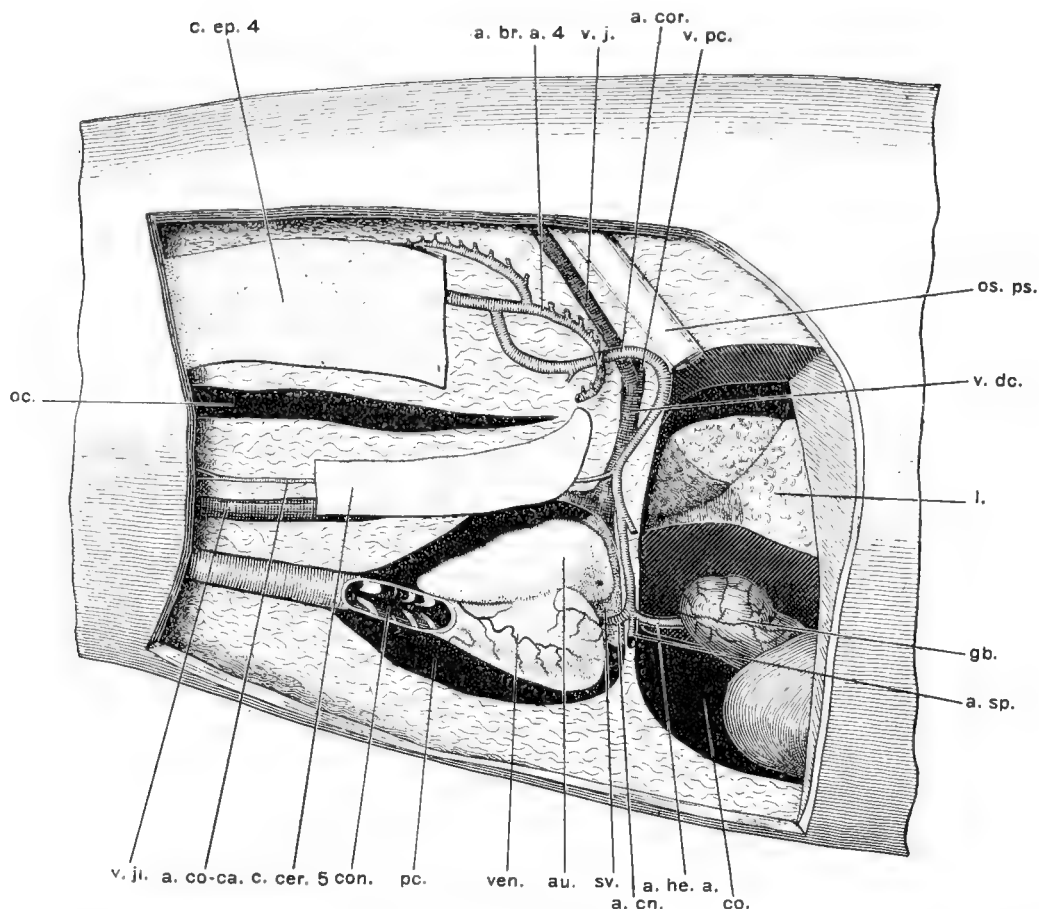


Fig. 11 Semi-schematic representation of the coronary artery and neighboring structures. In this specimen the left coronary artery is the one best developed.

a point which is emphasized by the fact that it sometimes anastomoses anteriorly with the fourth hypobranchial artery. Presumably there is such an anastomosis in the larvae studied by Allis ('11, l.c., p. 283). The main vessel enters the septum transversum where branches are given off to the region below the pericardium (*a.sp.*), to the liver (*a.he.a.*), and to the heart (*a.cn.*).

The artery which passes into the region ventral to the pericardium (fig. 12, *a.sp.*) sends forward several anterior branches which are chiefly distributed to the mm. pharyngo-claviculares and the posterior part of the sternohyoideus. There is also an anastomosing branch (fig. 12) connecting it with branches of the subelavian artery. The anastomosis is in the position of, and in a measure suggests, the coraco-hypobranchial anastomosis of the elasmobranchs as described especially by Pitzorno

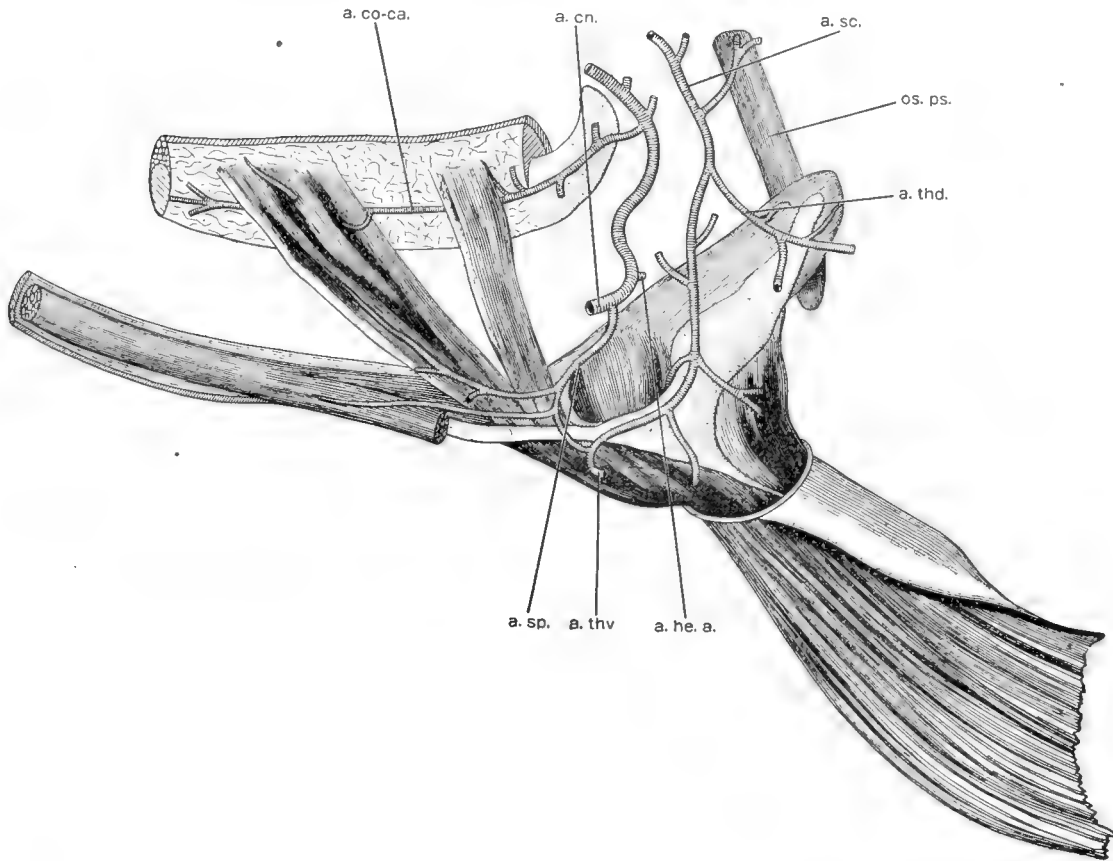


Fig. 12 Dissection to show the coronary and subclavian arteries

('05), but although the relationships of the subclavians are the same the other arteries are not entirely comparable. In several specimens the anastomosing vessel was apparently lacking.

The branches from the coronary artery to the liver may arise from both the right and left coronaries or from the larger one alone (figs. 11, 12, *a.he.a.*). These vessels, which may be designated as anterior hepatic arteries, are distributed to the whole anterior part of the liver. Their arrangement on entering the liver is fairly constant, there being two arteries in relation to each hepatic vein (fig. 13, *a.he.a.*). Within the liver they follow the veins and ramify in the tissues till lost to macroscopic methods. In some, possibly all, cases they supply the gall bladder (figs. 11, 13, *gb.*). In well injected specimens anastomoses are easily traced between the anterior and posterior (*a.he.p.*) hepatic arteries (fig. 13) but there is nothing to suggest that these are more than secondary connections. The writer does not know

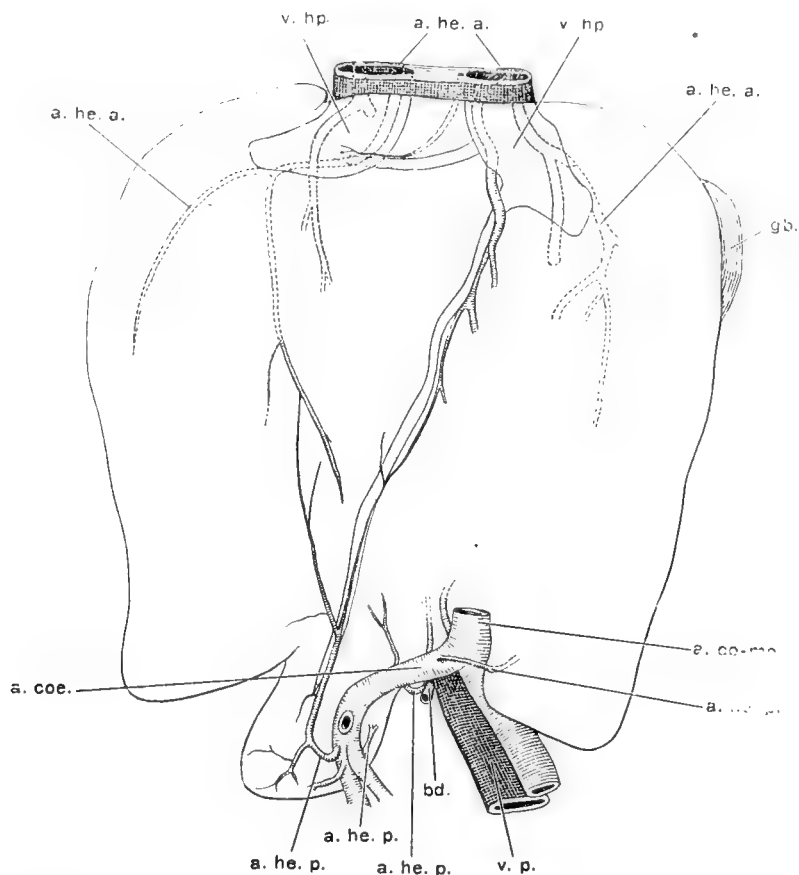


Fig. 13 Outline of liver showing main branches of anterior and posterior hepatic arteries.

of any other forms with vessels of this type and has no suggestion as to their possible homologies. They are not mentioned by either Allen or Allis in the papers cited.

The terminal branch of the 'coronary artery' (fig. 11, *a.cn.*) or the part which actually supplies the heart and seems to correspond to the posterior coronary of the skate, is generally, but not always, best developed on the right side. The smaller artery is, in all but exceptional cases, an insignificant vessel that runs toward the heart along the large coronary vein. The larger artery crosses the posterior part of the pericardial cavity in a free strand which also contains the small coronary vein. It passes under the auricle to the dorsal aspect of the ventricle (fig. 11) where, at a point slightly behind and to the right of the auriculoventricular junction, it breaks up into three or more branches. Like other arteries approaching their final distri-

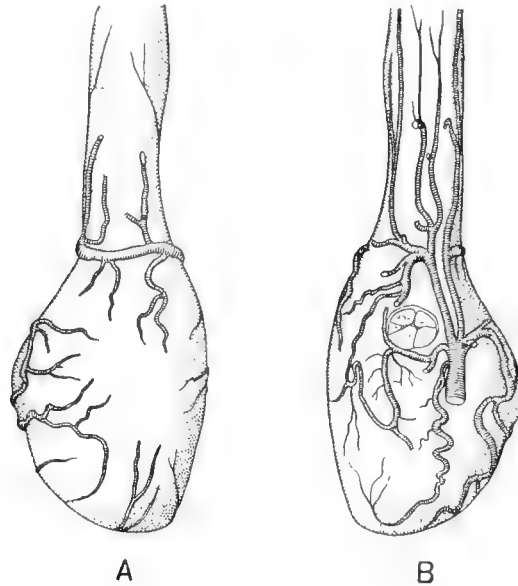


Fig. 14 Ventral (A) and dorsal (B) views of the ventricle showing distribution of the coronary artery. Branches with cut ends supply the investing lymphoid tissue.

bution it is extremely variable. Figure 14 is from sketches of the dorsal and ventral aspects of a typical ventricle. To bring the arteries into view the surrounding lymphoid masses were removed. Vessels represented with cut ends went to supply these structures.

The question as to the homology of the 'coronary artery' in *Polyodon* is not easily disposed of. The morphological importance of it depends on the value that may be attached to such structures as units. The vessels of fishes are variable to a marked degree and it does not seem difficult for new channels to be established nor is it surprising to find related forms more or less divergent in the matter of the distribution of minor arteries. Carazzi (op cit.) states that in working with a single form (*Scylium*) he finds in various individuals the arrangements described for different species and even different genera. He seems to be inclined to emphasize the functional rather than the morphological significance of the different plans. How far the divergent arteries of *Polyodon* are new developments or to what extent they are direct modifications of a more primitive condition cannot be judged with any assurance till their embryology is known.

ARTERIES OF THE PHARYNX

Each efferent branchial artery on approaching the roof of the pharynx leaves its groove in the epibranchial cartilage and crosses the anterior aspect of the *m. levator arcus branchialis* (*m. lev. 1*, fig. 7). Medially it turns ventrally and somewhat posteriorly around the muscle a little proximad of its insertion. The relations with the cartilages vary somewhat in the different arches (fig. 15). The first passes through a triangular space bounded dorso-medially by the parasphenoid bone, antero-laterally by the first pharyngobranchial, and postero-laterally by the anterior end of the first epibranchial and second pharyngobranchial cartilages. Within this triangular space it gives rise to the common carotid artery (*a.cc.*). Leaving the triangle it crosses the medial part of the second pharyngobranchial, making a groove in its ventral surface to gain the deep median excavation between the wings of the parasphenoid behind. It unites with the corresponding vessel of the other side in a plane passing near the middle of the third pharyngobranchials. The third pharyngobranchial does not reach the parasphenoid above and so a triangle corresponding to the one in front is not completely closed in behind. This allows the second efferent artery to slip back to a more posterior position as shown in the figure. It reaches the median vessel in the plane of the end of the third epibranchial cartilage. The third and fourth vessels likewise have migrated somewhat backwards. All of these arteries are forced to make a ventral dip around the knife-like edge of the parasphenoid. On reaching the mesial side of the wing of that bone they turn abruptly upward and backward to enter the median vessel on its ventral side. The arrangement of these vessels, each separately emptying directly into a single median dorsal aorta, is in accord with some of the other ganoids and simpler sharks and is seemingly more primitive than the radices aortae of higher sharks and teleosts.

Pharyngeal branches of the main trunks are not well developed. There are, however numerous small twigs (fig. 15) that supply the roof of the mouth and one prominent artery (*a.ph.*)

from the second efferent vessel which runs up over the wing of the parasphenoid to the cartilage of the occipital region, where it is distributed around the origin of the levator muscles and may possibly send twigs through into the cranial cavity or anastomose with others coming out. Allis describes this vessel as coming from the first efferent artery; presumably it may arise from either the first or second. Allis also describes a commissure between the third and fourth efferent arteries. No large vessels were found going back to the oesophagus.

THE COMMON CAROTID ARTERY

The common carotid (fig. 15, *a.cc.*) arises from the first efferent branchial artery just in front of the anterior epibranchial cartilage. It is a short vessel which runs diagonally forward and inward along the mesial aspect of the first pharyngobranchial, near the end of which it may be said to terminate by dividing into internal and external carotids. In the adult this division is a very unequal one, the internal carotid, which arises from the posterior and ventral side of the vessel, being so small that it was at first entirely overlooked or mistaken for one of the pharyngeal twigs that are given off in this region. The minute internal carotid will be described further on in connection with the artery from the pseudobranch; the distal continuation of the main trunk from the end of the common carotid is here described as the external carotid, even though the hypoöpercular and orbito-nasal arteries are also involved.

THE EXTERNAL CAROTID ARTERY

The external carotid (*a.ce.* figs. 15, 16), appearing as a prolongation of the common carotid, continues around the anterior end of the first pharyngobranchial cartilage in which it makes a shallow groove. Laterally it passes through a rather large foramen into a longitudinal channel, the facial canal of the basis cranii. Here it gives off a large branch (*a.hyo.*) which follows the hyomandibular nerve out posteriorly through the facial foramen. The canal is produced forward beyond the point

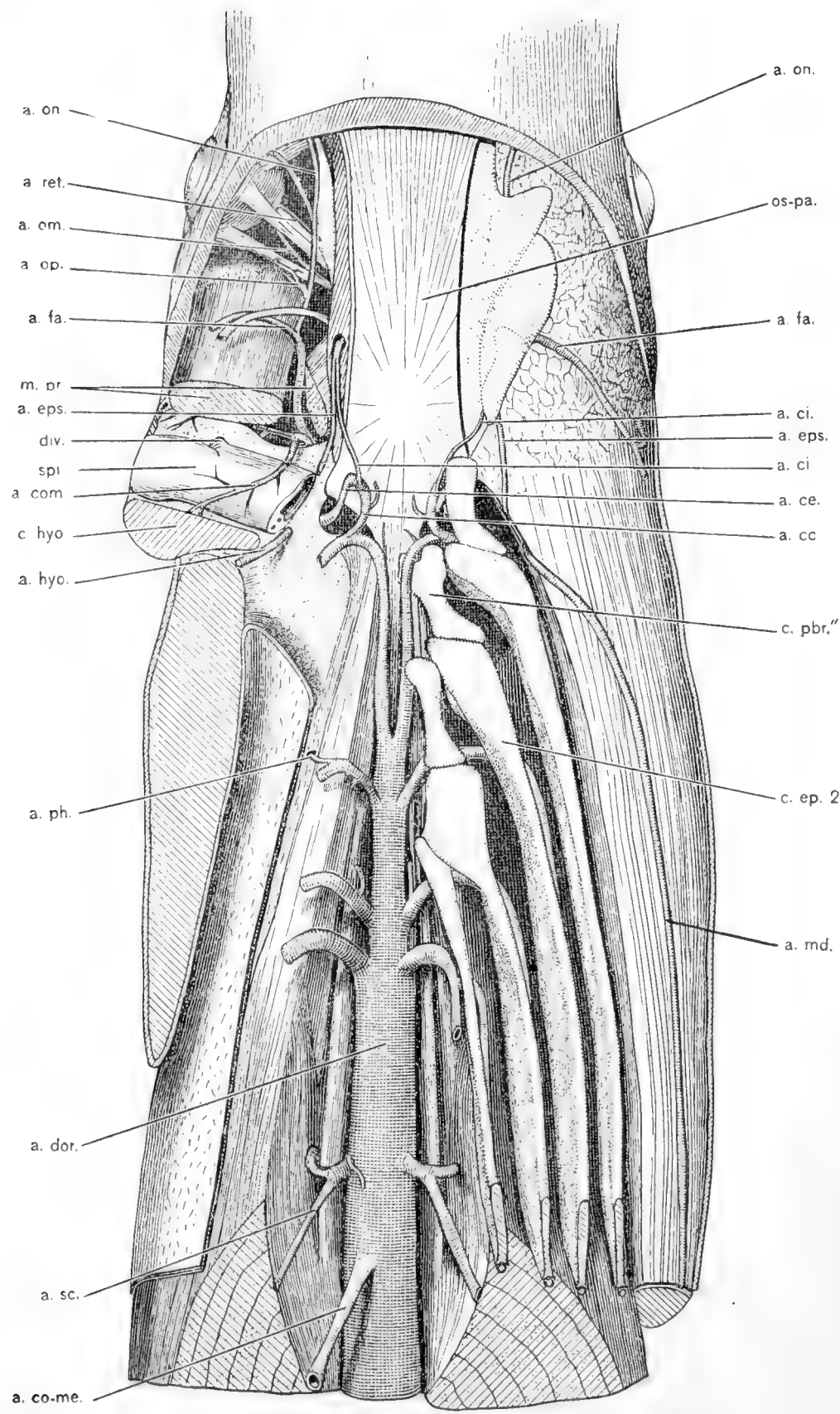


Fig. 15 Dissection of the roof of mouth and pharynx

where the hyomandibular nerve enters it and through this anterior part of the channel the artery again escapes from the cranial wall, emerging dorsal to the m. protractor hyomandibularis between its two slips of origin. As it leaves the canal it gives off a second branch. This, together with the first, probably represents the equivalent of the hypoöpercular artery of Silvester's descriptions (for teleosts) or the hypoöpercular and external carotid of *Amia* (Allis).

The posterior branch is here called the hyoöpercular (*a.hyo.*) and is likewise designated by Allis in his recent paper. Leaving the facial foramen along with the nerve, it ascends abruptly the posterior aspect of the hyomandibular bone, gives off a large muscular branch to the adductor hyomandibularis (fig. 16 *m.adh.*) and assumes a superficial position along the insertion of this muscle. Several small branches cross over to the anterior side of the hyomandibular bone and a very long one runs back under the operculum (*oper.*) to the inner side of the opercular flap. The main vessel, still following the hyomandibular nerve, traverses a groove in the distal end of the cartilage from which it sends a second branch to the opercular flap and then passes down under the branchiostegal ray (*br.*) to be distributed posterior and medial to the area supplied by the facial artery. For a further discussion of this artery the reader is referred to Allis's paper (l. c., p. 286).

The second branch of the external carotid immediately separates into two divisions. In the specimen described by Allis (l.c., p. 285) they arose separately from the main trunk. One runs laterally beneath the epithelium in front of the spiracular cleft and along the anterior face of the hyomandibular bone. The other divides into external and internal branches. The former (*a.com.*) ramifies over the cartilage around the spiracular cleft and diverticulum and supplies a nutrient branch to the anterior division of the protractor muscle. The latter (*a.fg.*) passes through the cartilage into a large 'fat-space' (the F-shaped groove of Bridge) beneath the frontal bone and medial to the hyomandibular articulation where it breaks up into small twigs.

Neither of the above arteries seems to fill completely the place of the external carotid of *Amia* although the second comes very near to it and, with the first, also covers the hyoöpercular of that form. The teleostean vessel which Silvester, endeavoring to follow Allis, called the hyoöpercular artery, but which, as Allis subsequently ('08 c) pointed out, is presumably comparable to the external carotid of *Amia*, finds a more or less complete homologue in the two vessels above described. The fact that they arise separately from a longitudinal trunk and not by a single stem from one of the several points where the 'hyoöpercular' ('external carotid') may appear in other forms, is probably of little morphological import. The branch (*a. com.*) from the anterior of these, which is described above as crossing beneath the epithelium of the spiracular cleft, was not found to connect directly with the spiracular vessel although it is strongly suggestive of the commissure that occurs here in *Amia*, the Loricati, and the teleosts described by Silvester.

The further continuation of the external carotid, apart from its proximal connection, is very much like the artery in teleosts which Silvester calls the external carotid and Allis ('08 c) the orbito-nasal. It is partly encircled by the *a. opthalmica magna* (fig. 15, *a.om.*) just as is the orbitonasal (Allis) in teleosts and, like that artery, it forms a large longitudinal trunk supplying branches to the orbit, eye muscles and nasal region. After leaving the facial canal and giving off its second branch as above described, it runs forward medial to the anterior portion of the protractor hyomandibularis already referred to. Along this part of the course there arise several small twigs probably of no morphological importance. As it approaches the mandibular nerve it turns somewhat laterad under the protractor mandibularis muscle where it gives off the large facial artery and then continues forward beneath the orbit and recti muscles. Allis (l. c., p. 285) describes the external carotid as terminating here by dividing into the 'orbito-nasal and maxillo-mandibularis arteries.'

The facial artery (figs. 15, 16, *a.fa.*) passes over the nerve to its anterior side and then follows it out, lying at first on the

ventral side of the protractor and then between that muscle above and the m. adductor mandibulae (*m.adm.*) below. In this position it gives off a large branch which immediately separates into posterior and anterior divisions. The former is a nutrient artery to the m. adductor mandibulae, but the latter, although supplying some muscular twigs, is distributed mostly to the abundant fatty tissues and follows the palatoquadrate forward to the median line. This recalls the vessel in *Polypterus* which arises from the internal carotid and has recently been considered by Allis ('08 b) as a possible remnant of the efferent mandibular artery of that form. Here it is not likely that it has any such significance. Further back the facial artery passes over the lateral aspect of the m. adductor mandibulae and in between the palatoquadrate cartilage and maxillary bone. Its rather numerous branches, some of them muscular, are indicated in figure 16. Below the angle of the mouth it still adheres to the lateral surface of the muscle as the latter gains its insertion on Meckel's cartilage. The main vessel finally becomes superficial ventrally by emerging from between the cartilage and overlying bone. From this point it may be traced forward along the cartilage nearly to the median line.

The next prominent branch (*a.op.*) from the external carotid enters the region back of the orbit, and is apparently the one designated by Allis as the ophthalmic branch. It gives a branch to the rectus muscles of the eye, just as does a similar vessel from the external carotid (orbito-nasal) of *Lopholatilus* and other teleosts (Silvester), and divides into two main branches. One of these supplies the fatty tissue behind and above the eye while the other goes to the similar tissue medial and anterior to the eye. Some of the terminal branches of the latter supply the m. obliquus superior and others reach the nasal region, being distributed apparently to a part of the sensory epithelium.

Two other branches (fig. 16) arise from the external carotid before it enters the rostrum. One of these runs ventral to the orbit, supplying the m. obliquus inferior and the superficial tissues below and in front of the orbit. The other has a somewhat similar but more anterior distribution except that its largest

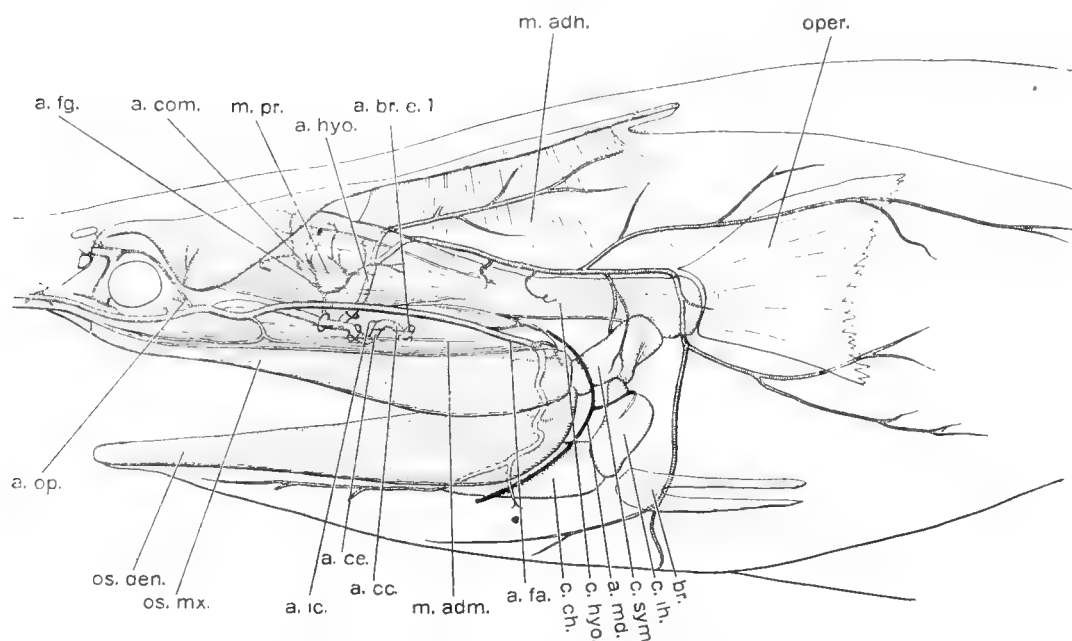


Fig. 16 Sketch of the external carotid and its branches. The deeper parts are indicated by lighter lines.

branch crosses the posterior face of the antorbital cartilage and then goes through a foramen into the nasal capsule. How far this perforating branch is homologous with the anterior end of the orbitonasal artery which perforates the antorbital process of teleosts it is difficult to say.

The artery of the rostrum (fig. 17), the terminal extremity of the external carotid, enters the snout by passing across a broad groove beneath the antorbital process. It then bends dorsally and, lying under the overhanging dorsal expansion of the central cartilaginous core, retains its individuality nearly to the anterior end. In its course through the rostrum it gives off a series of long medial vessels, which lie close to the cartilage except near their terminations where they may turn laterad, and another series of short lateral vessels, some of which actually arise on the medial side and then pass around the main stem dorsally. Through the greater part of its length the rostral cartilage is hollow and filled with fat behind and a kind of mucous tissue in front. The nutrient artery to this tissue arises from the main vessel shortly after the latter enters the rostrum. At its origin it bends somewhat caudad and then goes through a

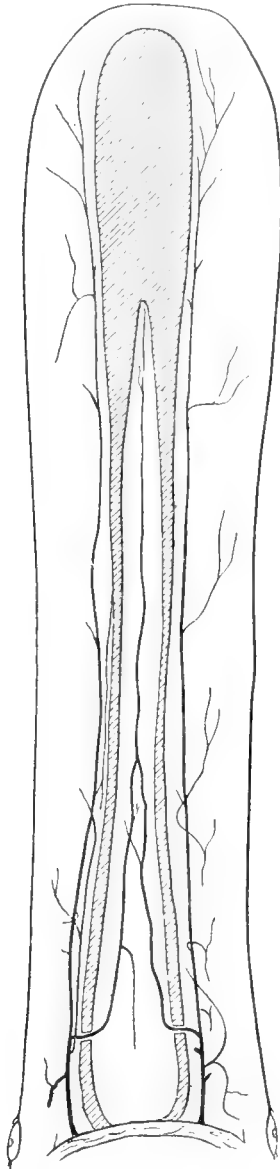


Fig. 17 Arterial supply of the rostrum. The cartilage is cross hatched.

foramen in the cartilage. The two nutrient arteries, one from either side, run obliquely forward and inward giving off anterior and posterior branches. The latter is deep and reaches the dorsal wall of the cartilage where possibly it sends through perforating branches. There are other perforating branches further forward. The main stems of these nutrient arteries finally fuse and the resulting median vessel is continued anteriorly through the mucous tissue. It ends finally in one or more perforating vessels.

Although the foregoing account shows quite clearly that the extensive system of vessels here described as the external carotid (fig. 16) and its branches is really much more than is usually embraced by that term, this designation is employed for convenience in the description of a natural system, the parts of which cannot be satisfactorily homologized by macroscopic methods alone. The several interesting questions that naturally arise in this connection must undoubtedly await embryological studies for their final solution.

INTERNAL CAROTID AND EFFERENT PSEUDOBANCHIAL ARTERIES

The internal carotid artery (fig. 15, *a.ci.*) is intimately connected with the efferent pseudobranchial (*a.eps.*). It at first runs anteriorly in the roof of the mouth, lying on the ventral surface of the parasphenoid bone. Anteriorly it turns laterally and, dorsal to an expansion of the bone, enters a canal in the basis cranii, where it is soon joined by the efferent pseudobranchial (*a.eps.*) which is the larger of the two. This vessel arises in the pseudobranch from about twenty filamentar arteries and, gaining the ventral surface of the protractor hyomandibularis, passes forward across that muscle to a canal which it enters at a point dorsal and anterior to the opening of the internal carotid canal. The canals unite in front and the arteries within anastomose. This anastomosis may apparently be effected through a large connecting branch or more commonly the two arteries completely fuse for a short distance. Anterior to the anastomosis the internal carotid becomes the larger vessel and the efferent pseudobranchial, now greatly reduced, proceeds as the *a. ophthalmica magna* (fig. 15, *a.om.*). This artery, while still in the cartilage, gives rise to several small twigs which could not be traced far, and then escapes from the cranial wall above the trigeminal nerve. It is at first posterior to the rectus muscles and then comes to lie in between them, following an independent course to the back of the eyeball which it enters just posterior to the entrance of the optic nerve. A few small twigs may be

given off on the outside of the sclerotic, some of them possibly anastomosing with the a. retinalis.

Returning to the internal carotid proper, we find it proceeding from its anastomosis with the efferent pseudobranchial artery through the basal cartilage into the cranial cavity. This region is carefully described by Allis (l.c., pp. 289-290). In this part of its course it gives off a long slender vessel, apparently confined entirely to the cartilage, which arches forward, ultimately reaching the median line. Just inside the cranium the a. retinalis (*a.ret.*) separates and accompanies the optic nerve to the eye. This is a very minute vessel which it is difficult to trace. Lateral to the diencephalon the internal carotid divides palmately into three branches, of which the posterior is largest, the anterior smallest. Allis apparently found only two of these, but he did not attempt to trace the encephalic arteries. The anterior division, which is sometimes greatly reduced, runs along the ventro-lateral side of the corpus striatum, dividing in no very constant manner into the twigs that supply this region. Its terminal rami run in among the coarse bundles of the olfactory nerve and one of them gains the dorsal side of the telencephalon. If the anterior division be reduced in size, as is often the case, its place is taken by a branch from one of the other two, either of which may send out a branch to cover practically the same region. The middle division of the encephalic artery, which, as just stated, often shows an anterior branch, ascends in the angle between the telencephalon and diencephalon. As it approaches the epiphyseal region it turns abruptly backward over the dome-shaped midbrain where it is joined by its fellow of the opposite side. There may be merely cross anastomoses between the two or they may fuse completely, in which case a single median vessel supplies the posterior aspect of the midbrain and gives off on either side a large branch which turns laterally and ventrally between the optic lobes and cerebellum. These arteries are subject to considerable variations and these variations are correlated for the most part with inverse variations in the lateral rami of the third encephalic artery with which they anastomose. The posterior and largest of the

encephalic arteries like the second may or may not give rise to a vessel to the corpus striatum. In one specimen, where such a branch was well developed, no anastomosis could be detected between it and the anterior artery by the gross methods employed. The main trunk turns medially between the inferior lobe and the anterior end of the medulla. As it arches backward it gives off one or two lateral branches which run up between the optic lobe and the cerebellum to anastomose as above described with the dorsal artery of the brain. The posterior arteries of the two sides join each other in the angle between the inferior lobes and the medulla, but from this point of union two vessels instead of one run caudally as far back as the level of the vagus nerve and may even extend further back before finally uniting. From these two parallel basilar arteries in front and their single posterior prolongation behind, a number of small branches are given off to the medulla, to the roots of the nerves, (nearly all of which are accompanied laterally by small arteries) and to the floor of the cranial cavity. Several small vessels of the latter group go to the optic capsule. In addition to these smaller branches there are on either side two large branches which seem to be constant in occurrence, but not in position. The more anterior of these runs laterally, either in front of the auditory nerve and between the roots of the V-VII complex, or posterior to all of these, to gain the lateral side of the medulla along which it runs caudally. The other and more posterior gives off a small branch and then follows the ninth nerve laterally as far as the auditory region where it turns aside to be distributed about the sacculus. No end to end connection between the basilar artery and the vertebral branch of the subclavian, which enters the spinal canal further caudad, could be detected, although it is not improbable that small anastomoses between the two arteries do occur. It is evident, at any rate, that the type of circulation, common to many of the vertebrates, including some of the fishes, in which the vertebral arteries are an important factor in the cerebral circulation, has not been attained by *Polyodon*.

It will be seen from the foregoing account that the relations of the internal carotid are rather more in accord with conditions in such other ganoids as *Amia* and *Acipenser* than is the case with the external carotid. The connection of the internal carotid with the efferent pseudobranchial and ophthalmica magna arteries is clearly explained in Allis's paper, and calls for no farther discussion here. It remains only to be said that in my larva, which is younger than those studied by Allis, the relative size of the internal carotid and the efferent pseudobranchial artery running beside it is very different from what it is in the adult. In a 74 mm. specimen the internal carotid, instead of being much the smaller vessel, is five or six times larger than the other. This would seem to indicate that in *Polyodon* the presence of an anterior carotid (i.e., one deriving its supply chiefly from the pseudobranch) is secondary and not the primary condition as it is said to be in sharks.

THE DORSAL AORTA

The origin of the dorsal aorta from the efferent branchial arteries has already been described. A few millimeters behind the entrance of the last pair of arteries the aortic wall becomes chondrified and the vessel from this point on is invested by a thick wall of cartilage which is interrupted only in the mid-dorsal line. It is decidedly flattened dorso-ventrally, so that in cross section it is in the form of a small sector of a large circle or even crescentic in outline. In surface view, when dissected out, the dorsal aspect shows regular metameric markings in the cartilage, but the ventral aspect is ridged and furrowed in a very irregular manner. A most striking feature in connection with the vessel is a longitudinal ligament (fig. 18, *lig.*), suspended in the lumen throughout its entire length. This ligament is attached in front and behind and supported throughout by a continuous thin membrane of fibrous tissue which binds it to the notochordal sheath along the non-chondrified dorsal line of the aortic wall. The flat suspensory portion is so deep that the

rounded part below rests on the ventral inside wall of the aorta, thereby dividing it into essentially distinct longitudinal compartments. In specimens fixed in a curved position the ligament stretched diagonally from side to side in accordance with the bends in the body. Perhaps, in addition to the function suggested by this condition, it may also act in opposition to the interspinous ligament. This peculiar structure was also observed by Bridge ('79), who suggested tentatively that it might represent the subchordal rod of elasmobranchs. I find a similar structure occurring in *Scaphirhynchus* and a rudiment of it in the trout and *Ameiurus*.

The branches of the dorsal aorta are an irregular series of segmental arteries, beginning with the subclavian in front and given off throughout the whole course; a series of short ventral branches to the air-bladder, gonad, and kidney; and the large coeliaco-mesenteric artery which arises at the anterior extremity of the coelom. The aorta itself terminates as it approaches the end of the tail by dividing into short lateral branches which pass out of the axial cartilage and again divide, this time into dorsal and ventral branches for the two lobes of the tail.

THE SEGMENTAL ARTERIES

There are two main divisions of the segmental arteries, the dorsal, or parietal, the ventral, which in the coelomic region are the splanchnic arteries. They may arise along the lateral triangle of the aorta by short common stems or, more frequently the dorsal and ventral divisions are quite distinct from each other. These vessels are somewhat irregular in their appearance and vary greatly in size. For the most part, however, there is one pair of good sized arteries for every two or three pair of nerves. Sometimes the two members of a pair will be very different in size and sometimes two successive arteries on the same side will both be large, especially in the region of a fin. Each dorsal parietal artery (fig. 18, *a. pa.*), on emerging from the aortic wall, sends a large horizontal branch diagonally out-

ward and backward in an intermuscular septum. This branch ramifies somewhat in the septum but maintains its individuality until it reaches the lateral line where it divides into dorsal and ventral branches and shows some inclination to anastomose with similar vessels in front and behind it. The main stem next gives off a spray of small vessels to the notochordal sheath and higher up a branch that enters the spinal canal. Beside the neural spine there arises a second lateral branch very much

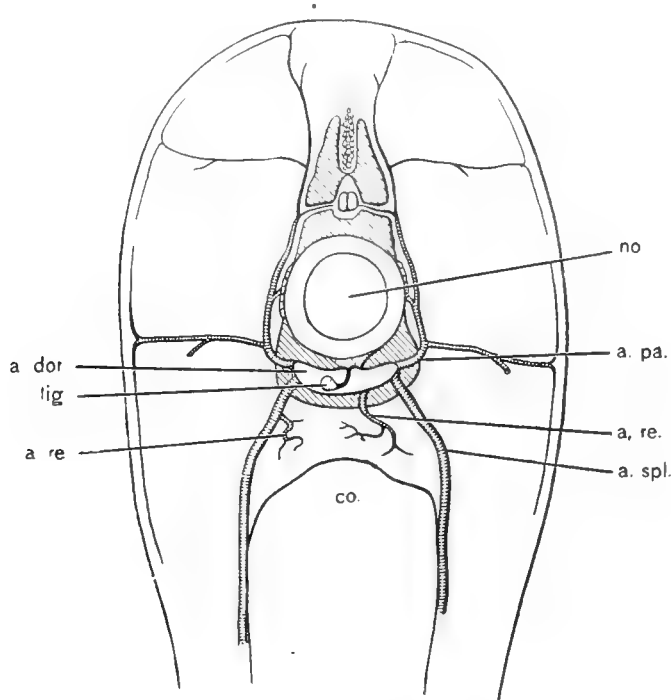


Fig. 18 Diagramatic cross section in the region of the kidney

like the first. Dorsally the parietal artery itself ends in a terminal bifurcation, or, in the region of the dorsal fin, becomes one of the arteries of supply to that structure. In a fish about a meter long there were five pairs of dorsal arteries to the fin.

The splanchnic arteries (fig. 18, *a. spl.*) may give off small ventral branches to the air-bladder, kidney or gonad, lateral to which they continue ventrally close to the peritoneum. They show a slight tendency to anastomose and form longitudinal connecting vessels below. Several of them supply each ventral

fin and in some cases at least, terminal branches around the anus run forward on the rectum to become continuous or to anastomose with the mesenteric artery. Posterior to the coelom the vessels comparable to these partake more of the character of the parietal arteries. About eight or nine pairs of them supply the anal fin.

The subclavian arteries (figs. 12, 15, *a. sc.*) have the general relations of the other segmental arteries. They arise at a considerable distance behind and entirely independent of the fourth efferent branchial arteries. Each at once gives off the characteristic dorsal branch which ramifies in the occipital region and supplies the neural canal but was not found to anastomose directly with the basilar artery. The lateral division runs ventrad and laterad behind the jugular vein and in front of the post scapular. Aside from the anterior branches already described in connection with the coronary and hypobranchial arteries, it gives off posteriorly above the scapular a long superficial branch (*a. thd.*) which is probably to be identified with the a. thoracico-dorsalis of the selachians (Pitzorno, loc. cit.), and a ventral branch (*a. thv.*) which is probably the a. thoracico-ventralis of those forms. Its principal remaining branches supply the musculature of the fin.

ARTERIES TO THE KIDNEY

The arteries that directly supply the dorsal side of the kidney (fig. 18, *a.re.*) and swim-bladder are numerous small vessels that arise irregularly along the ventral side of the aorta. Within the kidney there is also sometimes developed a longitudinal stem of greater size. The finer anatomy of these vessels in relation to the renal tissues was not studied.

THE COELIACO-MESENTERIC ARTERY

The coeliaco-mesenteric artery (figs. 15, 19, *a. co me.*) arises from the dorsal aorta between the levels of the anterior end of the coelom and the posterior margin of the pronephros. Enclosed

in a free strand of tissue, it descends along the anterior face of the air-bladder nearly to the cardiac end of the oesophagus, a distance of about a decimeter in a fish a meter long. Throughout the greater part of this portion of its course it is closely enveloped between the air-bladder and the dorsal aspect of the right lobe of the liver, making a deep furrow in the later. On the right side near the junction of the oesophagus and stomach it gives off its first large branch, the coeliac division (figs. 13, 19, *a. coe.*), and comes into relation with the portal vein. Associated with the vein in a free strand, it passes under the oesophagus to the right side of the intestine to which it gives a few branches and then enters the spleen which it supplies copiously. Emerging behind, dorsally and somewhat to the right it gives off a few small branches which go to the rectum, and a large one which enters the mesentery and bifurcates, one division going to the fundus of the stomach and the other to the posterior ventral aspect of the air-bladder where it anastomoses with the dorsal arteries to that organ. This casual anastomosis may become important for, in one specimen, the main blood supply to the whole posterior part of the alimentary canal came down through the channel thus opened up, making a kind of anomalous posterior mesenteric artery. Behind the spleen the main vessel (*a. rec.*) is continued in the median line down the dorsal side of the rectum to which it gives off several branches, and, at least in some cases, becomes directly continuous and also anastomoses indirectly with some of the posterior ventral segmental arteries.

The coeliac division of the artery gives rise first to oesophageal branches, some of the anterior of which may send twigs to the swim-bladder above and the liver below. At about the same level there also arises a slender artery (*a. he. p.*) to the liver which often reaches as far as the gall-bladder. This is the principal right posterior hepatic artery. The main vessel, subject to considerable variation, gives rise to branches to all the neighboring organs and then turns dorsally in the angle on the right side of the gastro-intestinal junction. As it approaches the

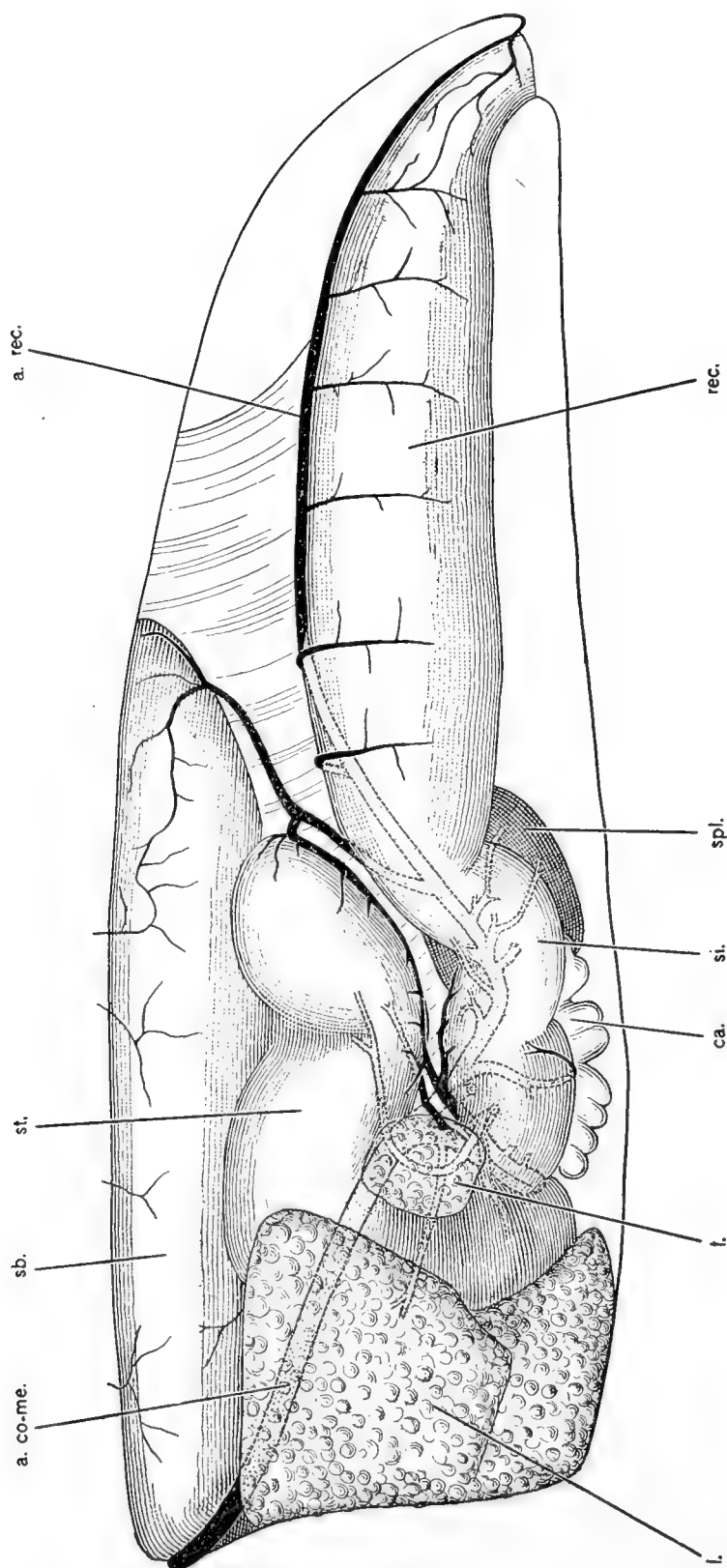


Fig. 19 Schema of the coeliaco-mesenteric system

mouth of the pyloric gland it bifurcates. The anterior division branches palmately into a number of arteries to the stomach, a left posterior hepatic artery, which, as previously stated, anastomoses with the anterior hepatics, and a large artery to the anterior part of the pyloric gland. The posterior division breaks up into three branches, one of which supplies the posterior part of the pyloric caeca, one to the small intestine and one to the side of the stomach.

As is the case with many other forms the details of distribution vary greatly with all of these arteries, and a wide latitude must be allowed for individual differences.

CONCLUSION

The foregoing account of the general anatomy of the arterial system of Polyodon shows that, while in many respects the ganoid type, especially as exemplified by *Acipenser*, is quite clearly indicated, there are, nevertheless, some features which suggest elasmobranch and teleostean conditions. Among the former may be mentioned the posterior coronary arteries which to some extent resemble those of the skates, and among the latter the orbitonasal artery of teleosts, although it must be confessed that the homologies of this vessel are by no means clearly established. Marked peculiarities of Polyodon, apparently not common to other fish so far as known, are the absence of anterior coronary arteries, the origin of the posterior coronary in the epibranchial region, and the existence of anterior hepatic arteries. The evidence of these characters, so far as it goes, indicates that Polyodon is not very closely related to any of the other forms. Whether its resemblance to the skates, both here and in some of its skeletal structures, is anything more than a chance parallelism may well be doubted.

There is a close agreement between the account given here and that of Allis's paper wherever the two overlap. Small differences in regard to number and origin of vessels fall easily within the limits of individual variation which, in Polyodon, is very great.

The many differences, however, which Allis records as existing between his larvae and drawings which he has of adults can hardly be due, as he supposes, to immaturity of the former since all of the possible larval characters which he mentions are common to fish a meter or more in length.

LITERATURE

- ALLEN, WM. F. 1907 Distribution of the subcutaneous vessels in the head region of the ganoids, *Polyodon* and *Lepisosteus*. *Proc. Wash. Acad. Sci.*, vol. 9, pp. 79-125.
- ALLIS, E. P. 1897 The cranial muscles and cranial and first spinal nerves in *Amia calva*. *Jour. Morph.*, vol. 12, pp. 487-808.
- 1900 The pseudobranchial circulation in *Amia calva*. *Zool. Jahrb. Abt. Anat.*, Bd. 14, pp. 107-134.
- 1908 a The pseudobranchial and carotid arteries in *Ameiurus*. *Anat. Anz.*, Bd. 33, pp. 256-270.
- 1908 b The pseudobranchial and carotid arteries in *Polypterus*. *Anat. Anz.*, Bd. 33, pp. 217-227.
- 1908 c The pseudobranchial and carotid arteries in the gnathostome fishes. *Zool. Jahrb., Abt. Anat.* Bd. 27, pp. 103-104.
- 1911 The pseudobranchial and carotid arteries in *Polyodon spathula*. *Anat. Anz.*, Bd. 39, pp. 257-262, 282-293.
- AYERS, H. 1889 The morphology of the carotids, based on a study of the blood vessels of *Chlamydoselachus anguineus*, Garman. *Bull. Mus. Comp-Zool. Harvard College*, vol. 17, pp. 191-223.
- BOAS, J. E. V. 1880 a Über Herz und Arterienbogen bei *Ceratodus* und *Protopterus*. *Morph. Jahrb.*, Bd. 6, pp. 321-353.
- 1880 b Über den Conus arteriosus bei *Buterinus* und bei anderen Knochenfischen. *Ibid*, pp. 527-533.
- BRIDGE, T. W. 1879 On the osteology of *Polyodon folium*. *Phil. Trans. Roy. Soc. London*, vol. 179, pp. 683-733.
- CARAZZI, D. 1904 Sulla circolazione arteriosa cardiaca ed esofagea della *Scyllium catulus*. *Intern. Monatschr. Anat., Physiol.* Bd. 21, pp. 1-20.
- CAVALIÉ, M. 1904 La vésicule biliaire et sa circulation artérielle, chez quelques poissons de mer (*Torpedo galvani*, *Scyllium catulus*, *Galeus canis*.) *C. R. Soc. Biol. Paris*, T. 55, pp. 1386-1388.
- DANFORTH, C. H. 1911 A 74 mm. *Polyodon*. *Biol. Bull.*, vol. 20, pp. 201-204.
- FERGUSON, J. S. 1911 The anatomy of the thyroid gland of Elasmobranchs, with remarks upon the hypobranchial circulation of these fishes. *Amer. Jour. Anat.*, vol. 11, pp. 151-208.
- GREIL, A. 1903 Ueber die Entwicklung des Truncus arteriosus der Anamnia. *Verh. Anat. Ges.*, Bd. 17, pp. 91-105.
- GUDERNATSCH, J. F. 1911 The thyroid gland of the Teleosts. *Jour. Morph.*, vol. 21, no. 4, Supplement, pp. 709-782.
- HYRTL, J. 1858 Das Arterielle Gefässsystem der Rochen. *Denkschr. d. Kais. Akad. d. Wissens. Wien*, Bd. 15.

- JORDAN, D. S. 1899 A manual of the vertebrate animals of the northern United States. Chicago. Eighth ed. p. 33.
- MAURER, F. 1888 Die Kiemen und ihre Gefäße bei anuren und urodelen Amphibien, und die Umbildungen der beiden ersten Arterienbogen bei Teleostiern. *Morph. Jahrb.*, Bd. 14, pp. 175-222.
- PARKER, G. H. 1900 Note on the blood vessels of the heart in the sunfish (*Orthogoriscus mola* Linn.). *Anat. Anz.*, Bd. 17, pp. 313-316.
- PARKER, G. H., and DAVIS, F. K. 1899 The blood vessels of the heart in *Characharias*, *Raja*, and *Amia*. *Proc. Boston. Soc. Nat. Hist.*, vol. 29, pp. 163-178.
- PARKER, T. J. 1884 A course in zootomy. London.
- 1886 On the blood-vessels of *Mustelus antarticus*. *Philos. Trans. Roy. Soc.*, London, vol. 177.
- PITZORNO, M. 1905 Ricerche di morphologia comparata sopra le arterie succlavia ed ascellare Selachii. *Monit. Zool. Ital.*, vol. 16, pp. 94-103.
- SENIOR, H. D. 1907 a The conus arteriosus in *Tarpon atlanticus* (Cuvier and Valenciennes). *Biol. Bull.*, vol. 12, pp. 146-151.
- 1907, b Note on the conus arteriosus of *Megalops cyprinoides* (Broussonet). *Biol. Bull.*, vol. 12, pp. 378-379.
- SILVESTER, C. F. 1904 The blood-vesicular system of the tile-fish, *Lopholatilus chamaeleonticeps*. *Bull. Bureau Fisheries*, vol 24, pp. 89-144.
- STÖHR, P. 1876 Ueber den Klappenapparat im Conus arteriosus der Selachier und Ganoiden. *Morph. Jahrb.*, Bd. 2, pp. 197-228.
- WIJHE, J. W. van. 1882 Ueber das Visceralskelett und die Nerven des Kopfes der Ganoiden und von *Ceratodus*. *Niederl. Archiv Zool.*, Bd. 5.
- WRIGHT, R. R. 1885 On the hyomandibular clefts and pseudobranchs of *Lepidosteus* and *Amia*. *Journ. Anat. and Physiol.*, vol 19.

THE EMBRYOLOGY OF CRYPTOBRANCHUS ALLEGHENIENSIS, INCLUDING COMPARISONS WITH SOME OTHER VERTEBRATES

II. GENERAL EMBRYONIC AND LARVAL DEVELOPMENT, WITH SPECIAL REFERENCE TO EXTERNAL FEATURES¹

BERTRAM G. SMITH

From the Zoological Laboratory of Columbia University

TWO HUNDRED AND TWENTY-THREE FIGURES (EIGHT PLATES)

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VI. INTRODUCTION

The present contribution is one of a series of papers on the embryology of *Cryptobranchus*, of which Part I, dealing with the breeding habits, ovogenesis, maturation and fertilization, has already been published (Smith '12). In the preparation of

¹ Part I was published in the *Journal of Morphology*, vol. 23, no. 1, March, 1912.

this paper I am greatly indebted to Prof. Bashford Dean, under whose guidance it has been brought to completion.

During the past seven years the collection of an abundance of material has enabled me to preserve an ample supply in every stage of development. At least fifteen thousand embryos have been secured from nests, and nearly as many more have been obtained by artificial fertilization.

For convenience in description, the embryonic and larval history has been divided into stages, based chiefly on external characters. In making the division, the usual difficulty has been encountered, that the rate of development of each structure varies more or less in different embryos. In determining what shall constitute the interval between stages, the guiding principle has been to establish stages only so far apart that individual variations in the rate of development of the most important characteristics selected as criteria for classification shall not overlap. For purposes of more intensive study, each stage may be divided into tenths; this device is useful in following any single character or set of characters. Since development is a continuous process, the importance of studying a close series cannot be too strongly emphasized.

As an aid to obtaining the exact sequence of events, stress has been laid on the study of a series of stages preserved at short intervals, from a single lot of eggs fertilized at the same time. Every period of the embryonic development has been covered repeatedly in this way, an entire spawning of eggs being sometimes used in the study of three or four stages as distinguished in this account. Thus not only a close series, but a large number of embryos in each stage, representing several different spawnings, have been studied, so that the typical course of development could readily be distinguished from variations or abnormalities.

Moreover, the entire embryonic and larval history has been carefully followed in living material, repeatedly and for the most part with embryos freshly collected. The study of living material is of especial importance in the late cleavage and gastrula stages of *Cryptobranchus*; for in these stages the translucent condition of parts of the unpigmented embryo enables one to

gain a fair idea of what is going on inside. The ciliation of the ectoderm of the late embryo, and some features of the circulatory system, are best studied in living material.

An accurate and complete time record (Section X) of the course of development has been obtained by comparison of many different records of material kept alive during long periods of time; these results were checked by observing the rate of development of material freshly collected. One lot of embryos, collected in the fall of 1906 at the time of the closure of the neural folds, were kept alive in the Zoological Laboratory of the University of Michigan throughout the entire larval period, and their metamorphosis was observed at the end of the second year after fertilization. Specimens were preserved at intervals; shortly after metamorphosis the half-dozen remaining individuals died from causes unknown. Another lot of embryos collected in an advanced gastrula stage in the fall of 1910, were kept alive and in good condition in the Zoological Laboratory of the University of Wisconsin until May, 1911, when the last ones were preserved.

The study of external and internal structure has gone hand in hand, except for the post-gastrula stages; here, doubtful points in the interpretation of the external structure have in most cases been investigated by reference to serial sections.

In preparing the illustrations, composite or ideal figures have been avoided. Each drawing, unless otherwise specifically stated, is a faithful representation of an individual embryo; a sufficient variety of figures has been given to illustrate the most important deviations from the condition regarded as typical. All the drawings are the work of the author except figures 268 to 276 which were drawn by Prof. Bashford Dean and with his generous permission are here published for the first time; figure 203 which was kindly contributed by Dr. L. Hussakoff of the American Museum of Natural History; and figures 277 to 279 which were drawn by Miss Hedge of Columbia University.

The histological technique employed has already been given in Part I; it remains to record the methods used in photography. For embryonic stages, fixation in Solution B (see Part I) followed

by preservation in formalin, gives the best photographic results. While being photographed, the objects were immersed in water or formalin. Living larvae were anesthetized with chloretone. In all cases the exposure was made by daylight.

All the photographs with a magnification of $\times 4$ were made with a Bausch and Lomb Zeiss Tessar 72 mm. lens, fitted to a long bellows Pony Premo No. 6 camera. The camera was fastened in an erect position by means of an improvised wooden frame. Figures 262, 264, 265 and 266 were taken with a Zeiss Unar Lens; figures 263 and 267 with a Zeiss Apochromatic Planar. Seed's Non-halation plates were used throughout; they were developed with Adurol.

All the negatives are the work of the author except figure 266 which was made by Miss Frances J. Dunbar of the University of Michigan.

The photographs are untouched, except in a very few cases for the purpose of correcting slight defects in the negatives.

VII. CLEAVAGE

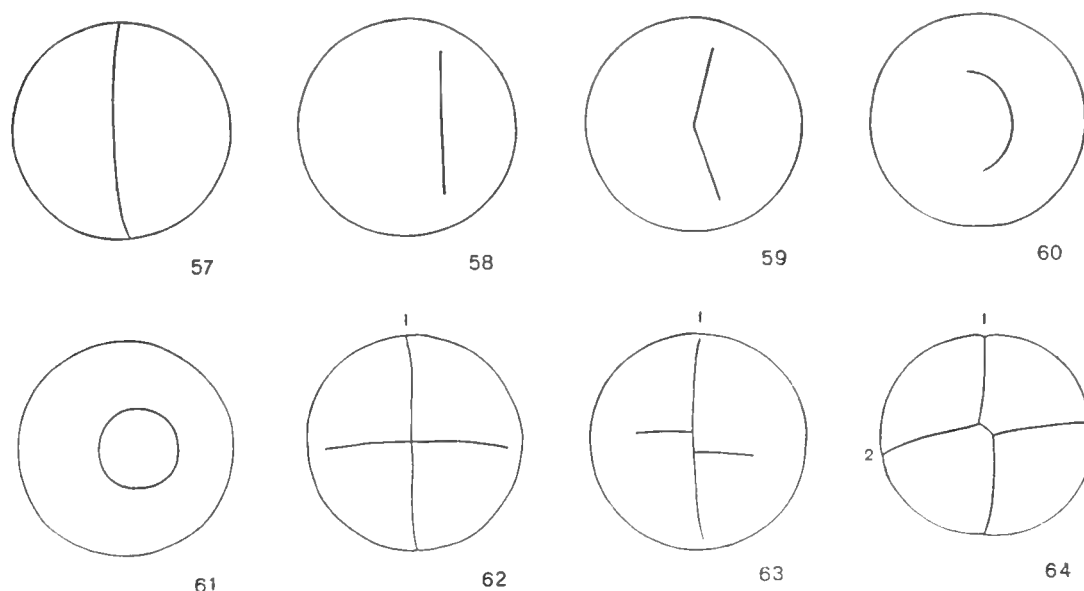
A. Description of cleavage by stages

Stage 1: (figs. 57 to 61 and 204). This stage is characterized by the presence of the first cleavage furrow only. The germinal area reaches nearly to the equator.

In artificially fertilized eggs the first cleavage furrow ordinarily appears about twenty-four hours after fertilization; the time may vary from eighteen to twenty-eight hours. The furrow begins as a pit, usually at the animal pole; it lengthens rapidly at first, then more slowly as it invades the regions of the egg more heavily laden with yolk. After the first cleavage furrow is well established, it becomes narrow in its middle portion, while still broad at the ends. The first cleavage furrow of the typical form becomes superficially complete in Stage 3 (third cleavage).

In the typical condition, the first cleavage furrow passes through the animal pole, and the division is equal (figs. 57 and 204). Variations from this condition occur. To test the amount of

variation, sixty eggs from a single spawning were examined in the first cleavage stage. In forty-eight cases the condition was of the typical character described above. In seven cases the first cleavage furrow was straight, but the cleavage unequal; figure 58 represents the extreme of this condition. In three cases, the first cleavage furrow passed through the animal pole, but its halves met at this pole to form an obtuse angle (fig. 59). In three cases, the first cleavage furrow passed through the animal pole, but its halves met at this pole to form an obtuse angle (fig. 59).



Figs. 57 to 64 Types of first and second cleavage of *Cryptobranchus allegheniensis*. $\times 3\frac{1}{2}$.

Fig. 57 The first cleavage furrow extends just to the equator, a little further than is usual before the appearance of the second furrow.

Fig. 62 The first cleavage furrow extends a little below the equator.

Fig. 64 The first cleavage furrow extends a little below the equator; the second furrow extends just to the equator.

In two cases the first cleavage furrow formed a semicircle about the animal pole (fig. 60). In two cases from different spawnings, neither of which furnished the material for the above data, circular first cleavage (fig. 61) was found; in each case the animal pole was excentrically situated within the area bounded by the cleavage furrow.

Goodale ('11) reports a case of circular first cleavage in *Speerperes*; the egg gave rise to a normal embryo. According to Eycleshymer ('04), in *Necturus* the cytoplasm is always unequally divided by the first cleavage, giving rise to blastomeres which in many cases are decidedly unequal.

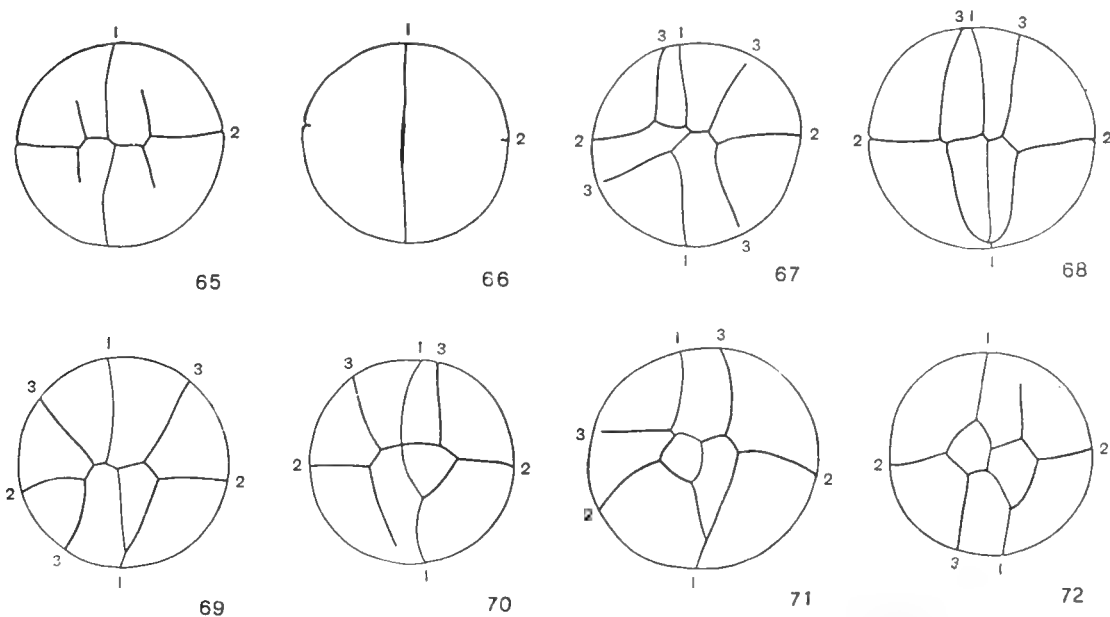
Stage 2: (figs. 62 to 64 and 205). The second cleavage furrow makes its appearance about six hours after the first, which by this time extends nearly to the equator of the egg. In the stage represented in figure 205, the first cleavage furrow has just reached the equator. The second cleavage furrow usually becomes superficially complete in Stage 4 (fourth cleavage), quite uniformly meeting the first cleavage furrow at right angles at the vegetal pole.

The earliest indication of the second cleavage furrow is usually a roughness in the region of the animal pole where the second groove is to intersect the first. The occurrence of 'Faltenkranzen'—a quivering of the surface with the formation of fine radiating or parallel wrinkles, which extend outward from the cleavage furrow—is quite marked at the time of the initiation of the second cleavage furrow. The cause of the formation of similar wrinkles in the frog's egg has been investigated by Charles B. Wilson ('96). For some time after its first appearance the second furrow is much broader, though of course shallower, than the first.

The second cleavage furrows usually depart from the same point as the first furrow, and proceed vertically, forming a single straight line at right angles to the first furrow (figs. 62 and 205). Occasionally the points of departure of the second furrows do not coincide, as shown in figures 63 and 64. The condition shown in figure 63 is rarely observed, and is transitional to that shown in figure 64, which is quite frequent. That portion of the first cleavage furrow lying between the points of departure of the second furrows may be called the polar furrow. As shown by the individual histories of a large number of eggs, in all cases in which a polar furrow is present the points of departure of the second cleavage furrows are separate from the beginning; the polar furrow at first exists as a part of the straight first cleavage furrow, but later becomes oblique through the shifting of cells. In no case in which the second cleavage furrows have their origin from the same point in the first cleavage furrow, has there ever been observed any shifting of cells during this or the following stage, of such a nature as to produce a polar furrow.

Comparison with the second cleavage of *Necturus* as figured by Eycleshymer ('04), Eycleshymer and Wilson ('10), and in some unpublished drawings of the living egg by Prof. Bashford Dean, leads to the conclusion that in *Necturus* there is much greater irregularity in the second cleavage than in *Cryptobranchus*.

Stage 3: (figs. 65 to 72 and 206). The third cleavage furrows appear about five hours after the beginning of the second; hence the interval is shorter than that between the first and second. At the time when the third furrows begin, the first furrow has usually reached or passed the equator.



Figs. 65 to 72 Types of third cleavage of *Cryptobranchus allegheniensis*. All the figures are of the upper hemisphere except figure 66 which represents the lower hemisphere of the egg shown in figure 65. In no case does any cleavage furrow except the first reach the lower pole. All the figures are camera drawings of preserved material. $\times 3\frac{1}{2}$.

As the cleavage furrows invade the more heavily yolk-laden lower hemisphere they become comparatively faint except at their extreme lower ends where they broaden out. During this stage the second and third cleavage furrows are in general broader than the first.

At the stage represented in figure 65, when the third furrows are well established but extend only a short distance from their

respective points of origin, the first cleavage furrow has reached the lower pole where its ends unite. The first cleavage furrow thus becomes superficially complete, thereby establishing the holoblastic character of the egg. That there is a strong meroblastic tendency is already apparent. In the region of the vegetal pole the first cleavage furrow is at first broad, but it later becomes narrow and faint.

With very few exceptions, the third cleavage furrows depart from the *second* furrow, at some little distance from its point of intersection with the first. In a previous paper (Smith '06) it was erroneously stated that the third cleavage furrows usually depart from the first furrow.

The third cleavage furrows ordinarily begin as two pits in the second furrow, equidistant from its point of intersection at the animal pole. From these two pits the third cleavage furrows ordinarily proceed in an approximately vertical direction (fig. 65), and do not become complete until a later stage (Stage 5).

From the time of the earliest appearance of the third cleavage furrows, the distances from the first cleavage furrow to their points of departure from the second remain unaltered; but the second cleavage furrow, originally straight, often becomes drawn into a zig-zag line, as shown in the figures.

As will be seen from a study of later stages, the third cleavage furrows rarely reach the vegetal pole, but as a rule extend obliquely in the lower hemisphere to join the *first* furrow at some distance from the lower pole (figs. 84 and 91 to 96). Hence the general statement may be made that the third cleavage furrows are intermediate between a true meridional and a true latitudinal cleavage but approach more nearly to the former type.

In the typical condition, the cleavage pattern has now lost its strictly radial, and acquired a biradial symmetry. I have purposely avoided the use of the word *bilateral* in this connection, not only because it does not fit the case so well as the word *biradial*, but in order to avoid the inference that the condition has anything to do with the bilateral symmetry of the future embryo. As will be seen by consulting the figures, this biradial condition of the cleavage pattern persists in the lower hemisphere through-

out the late cleavage stages, and in some eggs enables one to identify the early cleavage furrows even after the beginning of gastrulation.

Deviations from the type in Stage 3 show a series of conditions connecting the typical one with a true latitudinal third cleavage. In such cases the third cleavage furrow proceeds more obliquely, and at an earlier stage joins the first nearer the animal pole (figs. 68 to 72). In some cases one or more of the third cleavage furrows are truly latitudinal (figs. 70 to 72).

Rare cases occur in which a third cleavage furrow originates at the animal pole, or from a first cleavage furrow (see fig. 67 for an example of the latter case); occasionally, a third cleavage furrow may reach the vegetal pole, or unite with a second cleavage furrow near the vegetal pole (figs. 94 and 95).

In comparing the third cleavage pattern of *Cryptobranchus* with that of other forms, one of the most obvious generalizations brought out is that a vertical third cleavage is characteristic of heavily yolk-laden and highly telolecithal eggs: e.g., the squid (Watase '91); *Amia* (Dean '96, Whitman and Eycleshymer '97); *Lepidosteus* (Dean '95, Eycleshymer '99); *Acipenser* (Dean '95); *Ctenolabrus* (Agassiz and Whitman '84); *Serranus* (H. V. Wilson '91); *Ceratodus* (Semon '00 and '01); *Lepidosiren* (Kerr '00 and '09); *Cryptobranchus japonicus* (deBussy '04 and '05); and the pigeon (Blount '07). But the rule is not absolute; concerning the third cleavage of *Necturus*, Eycleshymer ('04) says: "In most cases the cleavage grooves are irregularly formed and it might be said that the variations are so numerous and so diverse that a special description must be written for each egg." From this statement and an inspection of his figures (see also Eycleshymer and Wilson '10), it appears that a type cannot be recognized for the third cleavage of this egg; that the irregularity is greater than in the case of *Cryptobranchus* and that there is a more marked tendency for the third cleavage furrows to come in latitudinally. My material for the very early cleavage stages of *Necturus* is too scanty to enable me to form any conclusion based on direct observations, but some unpublished figures of the early cleavage of *Necturus* drawn from the living egg by

Prof. Bashford Dean give confirmatory evidence of the irregularity of the third cleavage furrows. In *Desmognathus* the third cleavage furrows were vertical and regular in the few eggs studied by Wilder ('04); the third furrows depart from an earlier cleavage furrow at some distance from the animal pole. But Hilton ('04 and '09), who examined a considerable number of eggs of *Desmognathus* in this stage, states that this regular and vertical form of cleavage occurred in only two or three eggs; in the others the third cleavage was irregular. In *Diemyctylus* (Jordan '93) there is still greater irregularity in the third cleavage than is recorded for *Necturus* or *Desmognathus*: "With the completion of the second furrow all consistent regularity is at an end."

In eggs less heavily yolk-laden, as in *Amblystoma* (Eycleshymer '95) and the frog, the third cleavage is latitudinal.

Especially interesting from a comparative point of view are Budgett's observations on the cleavage of the crossopterygian *Polypterus* as given by Kerr ('07). "From Budgett's pen and ink sketch . . . we can see that the segmentation is at first characterized by its almost absolutely equal character. We may infer with considerable certainty that the two meridional furrows are succeeded by a latitudinal one which is practically equatorial." The egg of *Polypterus* is small, having a diameter of a little over one millimeter.

In urodeles we find a condition intermediate between the vertical third cleavage characteristic of the fishes generally, and the latitudinal third cleavage of the anura. In *Cryptobranchus* the vertical type prevails; in *Desmognathus*, *Necturus* and *Diemyctylus* there is increasing irregularity; in *Amblystoma* the third cleavage is latitudinal. The possible phylogenetic significance of the cleavage of *Cryptobranchus* will be considered later.

As a rather general rule, in eggs in which the third cleavage is usually vertical, the third furrows depart from the second rather than from the first or from the animal pole. As has already been seen in the case of *Cryptobranchus allegheniensis*, this rule is by no means absolute; but in general it applies also to the squid, and to the teleosts (e.g., *Ctenolabrus*, *Serranus*). DeBussy ('04 and '05) has described the cleavage stages of

Cryptobranchus japonicus; his material was meager and lacked first and second cleavage stages. He states ('05) that in the five eggs examined in the third cleavage stage, all the third furrows are approximately meridional. His figures ('04) represent the third furrows departing most frequently from the first cleavage furrow, sometimes from the second, sometimes from the animal pole. In the urodele *Hynobius* (Kunitomo '10) the cleavage pattern in this stage resembles that of *Cryptobranchus alleghe-niensis*, except that the third furrows do not so often depart from the second furrow. In *Amia*, Whitman and Eycleshymer ('97) describe the third cleavage furrow as follows:

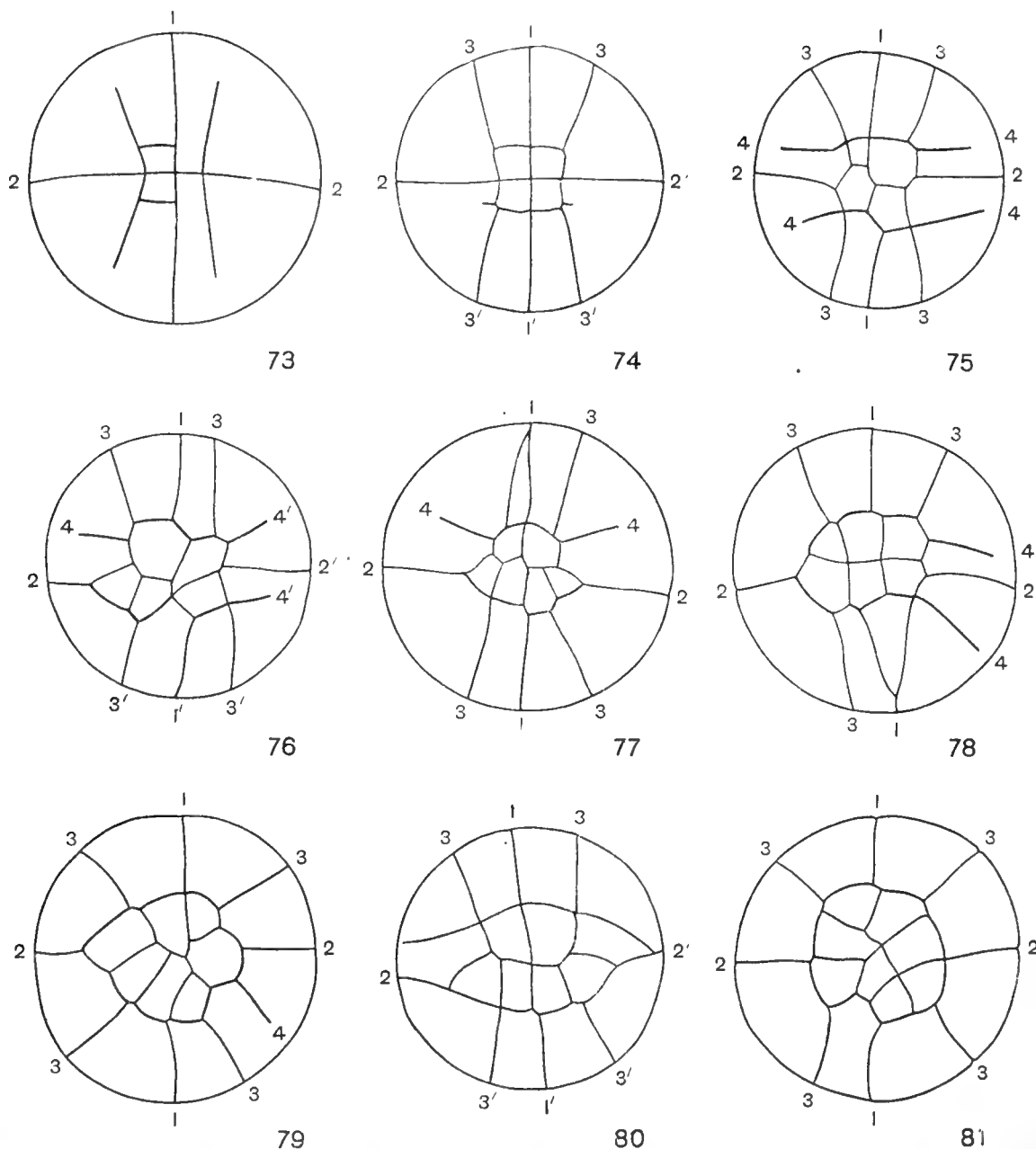
In the majority of cases they are vertical They generally all depart from one or the other of the first two meridionals, thus giving rise to a distinct bilateral appearance It oftens occurs that one or more of the set depart from the first meridional, while the rest depart from the second, or *vice versa*.

Stage 4: (figs. 73 to 84 and 207 to 209). This stage is characterized by the appearance of the fourth cleavage furrows, giving, when complete, sixteen cells. As will appear from the following observations, the number of micromeres is not constant, but varies from four to eight.

The fourth cleavage furrows appear about four hours after the beginning of the third, and about thirty-nine hours after fertilization. Ordinarily they begin as two grooves, cutting the *first* cleavage furrow at right angles, on each side of the second and a short distance from it (figs. 73 and 74). Thus in position and direction the fourth cleavage furrows alternate with the third, which cut the *second* at approximately right angles. The fourth cleavage is the first one to cut off micromeres from macromeres, and the division is very unequal.

In a given lot of eggs the sixteen-cell stage is reached quite uniformly at the same time, but with so much variation in the direction of the fourth cleavage furrows that at first sight no uniformity is recognizable. By the study of a large number of eggs the following generalizations are established:

(a) In each quadrant, one micromere is cut off between the first and second cleavage furrows, giving in each egg a minimum of four micromeres (fig. 74).



Figs. 73 to 81 Fourth cleavage of *Cryptobranchus allegheniensis*, $\times 4\frac{2}{3}$. All the figures are of the upper hemisphere. Figure 73 and 74 are drawn from the living egg; the others are camera drawings from preserved material. Figures 73 and 74 represent early stages of fourth cleavage; figure 80 is from the same egg shown in figure 74, representing the condition three hours later. Figure 81 is drawn from the egg photographed for figure 209.

(b) From the third cleavage furrows the remaining four parts of the fourth cleavage furrows may continue latitudinally, forming a complete circle or oval enclosing eight micromeres (fig. 81); or one or more of these four parts may continue approx-

imately parallel to the second cleavage furrow, extending vertically and increasing the number of macromeres instead of cutting off micromeres (figs. 75 to 80). Thus while the total number of cells is always sixteen, the number of micromeres varies from four to eight.

We thus get as one extreme type an approximately latitudinal fourth cleavage furrow; as the other extreme a fourth cleavage furrow divided into two separate grooves, one on each side of the second furrow and approximately parallel to it and to each other. Between these two extremes we find examples of all possible intermediate conditions.

With regard to the manner of fourth cleavage, eggs of this stage may be classified into five types, depending on the number of micromeres present. For the purpose of such a classification, irregularities in the third cleavage must be allowed for: in cases where the third cleavage has come in diagonally or latitudinally to cut off a small cell, such a cell is divided by the fourth cleavage into two small cells, of which only the one nearer the animal pole is to be counted as a micromere (fig. 77).

To determine the mode, twenty-five eggs were examined in the sixteen-cell stage, and the results tabulated as follows:

Number of micromeres.....	8	7	6	5	4
Number of cases.....	4	7	8	3	3

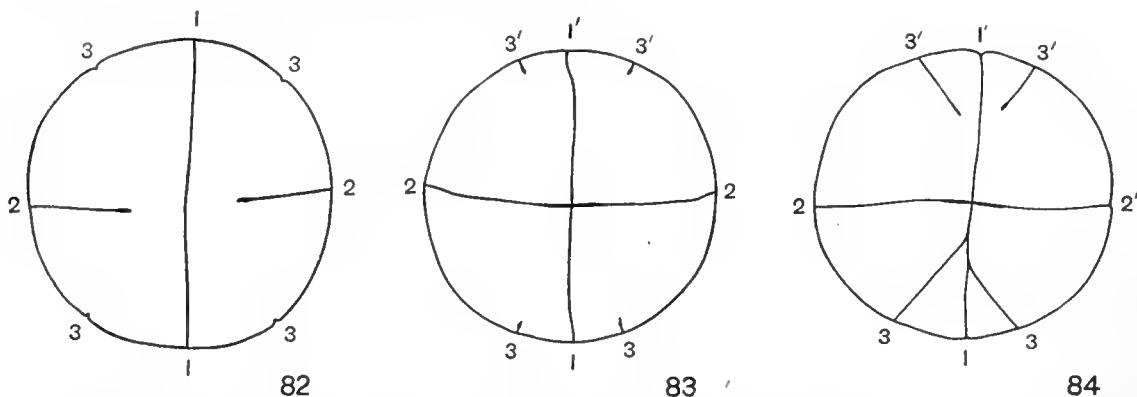
The table shows that the most frequent manner of cleavage is intermediate between the two extremes described.

In the majority of cases the micromeres are arranged with considerable regularity in two parallel rows, separated by the second cleavage furrow (see especially figs. 78 and 80). This is the necessary result of the biradial symmetry instituted by the normal mode of third cleavage, providing there is no extensive shifting of the micromeres. The condition reminds one of the cleavage pattern of the corresponding stage of the teleost egg.

Through a shifting of the micromeres, the biradial symmetry of the cleavage pattern of the blastodisc is usually interfered with (figs. 75 to 81). In this region, the first and second cleav-

age furrows become irregular and broken to an extent never observed in earlier stages. Outside of the region of the micromeres, the biradial pattern of cleavage is retained.

In this as in other cleavage stages the most recent furrows, and especially the most recent portions of such furrows, are in general quite noticeably the widest. This fact once established may be made use of in connection with other evidence to identify cleavage furrows. The broadening of the ends of the vertical furrows as they invade the lower hemisphere is a fairly constant feature of the cleavage; as shown by unpublished drawings from living material by Prof. Bashford Dean, it is also well expressed in the eggs of *Necturus*.



Figs 82 to 84 Lower hemispheres of fourth cleavage stages of *Cryptobranchus alleganiensis*. All the figures are camera drawings of preserved material. $\times 4\frac{2}{3}$.

Fig. 82 Lower hemisphere of the egg shown in figure 208.

Fig. 83 Lower hemisphere of the egg shown in figure 76. This figure would serve equally well to represent the lower hemisphere of the egg drawn for figure 75.

Fig. 84 Lower hemisphere of the egg represented in figure 80.

In the majority of eggs of this stage, the second cleavage furrows have reached the lower pole, and the third furrows have just passed the equator (figs. 82 to 84). The second cleavage furrows intersect the first at right angles at the lower pole. For some distance on each side of the pole the second cleavage furrows are for a time markedly wider than the first. The second furrows are further distinguished by the fact that the third furrows run closer to them than to the first. In the latter part of this stage the third furrows sometimes become complete (fig. 84), as a rule joining the first at some distance from the pole.

The biradial pattern of cleavage is thus preserved in the lower hemisphere, and throughout the later cleavage stages affords a trustworthy means of distinguishing first and second cleavage furrows in this region.

The fate of the fourth cleavage furrows that proceed vertically must be studied in later stages. They usually join the second furrow before reaching the lower pole (figs. 92 and 96).

In a given egg the micromeres vary somewhat in size; but a comparison of seventeen carefully drawn camera figures, and the examination of a large number of additional eggs, lead to the conclusion that in this stage there is no regularity in the distribution of large and small cells among the micromeres.

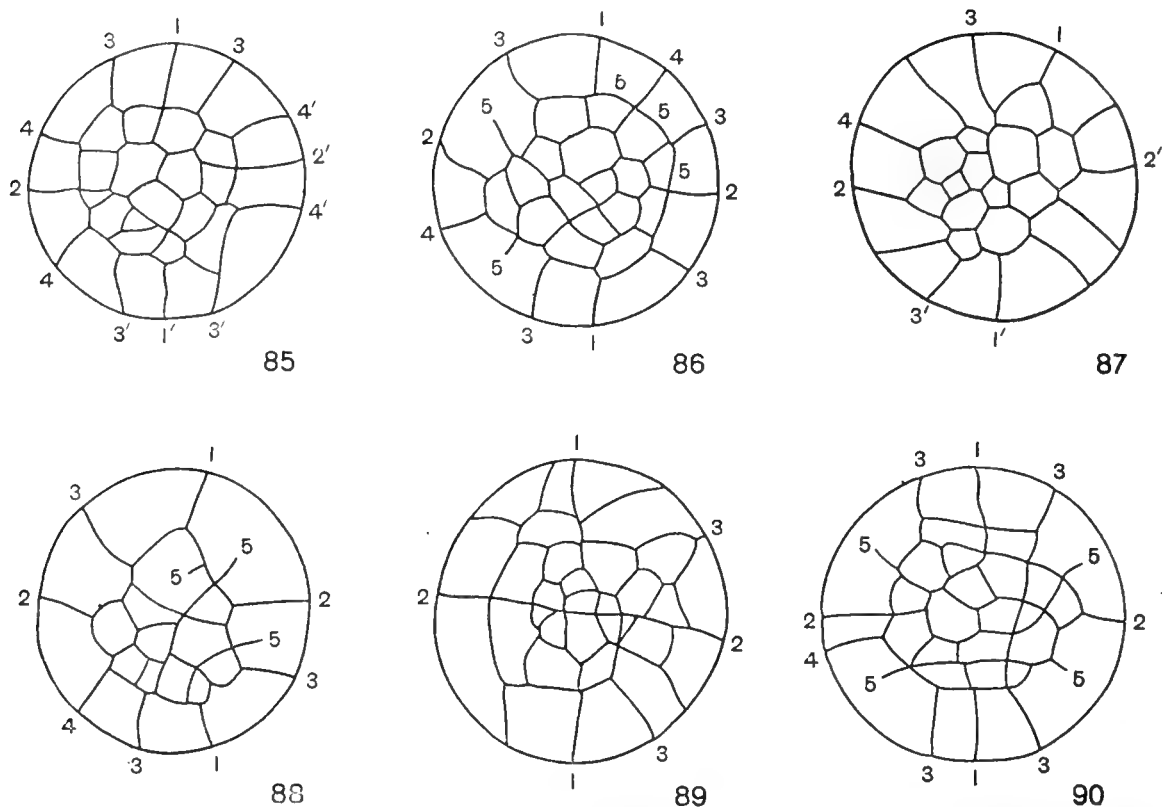
DeBussy's ('04) single figure of the fourth cleavage stage of *Cryptobranchus japonicus* shows six micromeres surrounded by an approximately circular cleavage furrow, and two recent furrows extending for a short distance vertically.

In *Desmognathus*, according to Wilder ('04), the fourth cleavage is latitudinal; this conclusion was based on the study of material very limited in amount. Hilton ('09) states that in a large number of eggs he has found only a few which exhibit so regular a type of cleavage as described in Wilder's eight cell and later stages.

In *Hynobius* (Kunitomo '10), the fourth cleavage furrows are more uniformly latitudinal than in *Cryptobranchus alleghe- niensis*. In *Necturus* (Eycleshymer '04; Eycleshymer and Wilson '10) and *Diemyctylus* (Jordan '93) a type is no longer recognizable.

In *Ceratodus* (Semon '00 and '01) the fourth cleavage is latitudinal. *Amia* (Dean '96; Whitman and Eycleshymer '97) and *Lepidosteus* (Dean '95; Eycleshymer '99) resemble the type with four micromeres described for *Cryptobranchus alleghe- niensis*.

Stage 5: (figs. 85 to 96; 210 and 211. This stage is reached about four hours later than Stage 4. It is characterized by the presence of the fifth cleavage furrows, giving a maximum of thirty-two cells, some incompletely divided. More than half of these cells are micromeres.



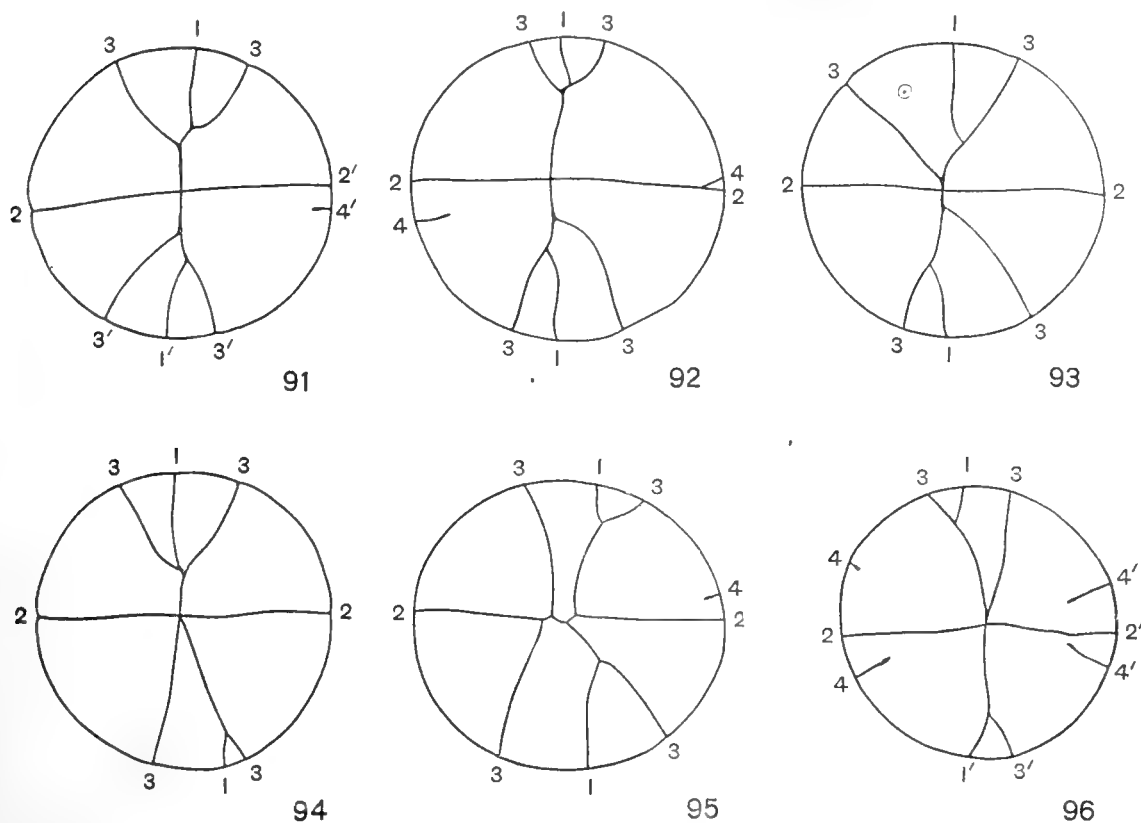
Figs. 85 to 90 Upper hemispheres of eggs of *Cryptobranchus allegheniensis* in the fifth cleavage stage. All the figures are camera drawings from preserved material. Figure 86 is drawn from the egg photographed for figure 211, and figure 87 from the egg photographed for figure 210. $\times 4\frac{2}{3}$.

The careful study of a large number of eggs emphasizes irregularity in this cleavage and the absence of a well established type. Two sets of fifth cleavage furrows are often recognizable: an inner, within the former region of micromeres, and an outer, just outside of this region. Either set may be, in whole or in part, vertical, latitudinal or oblique (see especially figure 85 for an example of outer, and figure 89 for an example of inner latitudinal cleavage furrows). A study of the most regular cases of cleavage described under Stage 6 shows that in these eggs the fifth cleavage furrows must have come in with greater regularity than in any eggs directly observed in the fifth cleavage stage: these fifth cleavage furrows are almost uniformly vertical, thus preserving the regular alternation in the direction of the furrows.

By the shifting of micromeres the biradial symmetry due to the manner of third cleavage is usually lost in the blastodisc, and unless the egg has been kept under continuous observation it becomes in most cases impossible to trace the first and second cleavage furrows entirely through the region of micromeres.

In preserved material, nuclei are visible from the surface in some of the micromeres of this and the following stages, indicating that these cells are becoming flattened out.

As already noted, in this stage if not in the preceding one, the third cleavage furrows become complete, usually joining the first at some distance from the pole (figs. 91 to 96). This apparent avoidance of the pole by the third cleavage furrow is doubt-



Figs. 91 to 96 Lower hemispheres of eggs of *Cryptobranchus allegheniensis* in the fifth cleavage stage. All the figures are camera drawings from preserved material. Figure 93 shows a persistent sperm pit (see Part I, Smith '12). $\times 4\frac{2}{3}$.

Fig. 91 Lower hemisphere of the egg whose upper hemisphere is shown in figure 87.

Fig. 96 Lower hemisphere of the egg whose upper hemisphere is shown in figure 85. The fourth cleavage furrows have extended further than is usual in eggs of this stage.

less the mechanical result of the location of the earlier course of the third furrows nearer to the first than to the second; they swerve from the vertical toward the nearest existing cleavage furrow. An analogous pattern may sometimes be observed in the cracking of the corners of a section of cement walk.

I have observed this biradial cleavage pattern in corresponding stages of the lower hemisphere of occasional eggs of *Necturus*; it is clearly expressed in the cleavage of *Desmognathus* as figured by Wilder ('04) and Hilton ('04 and '09).

The same tendency to join the nearest existing vertical furrow is shown by those fourth cleavage furrows, as a rule not yet complete, that come in vertically. They usually join the second furrow, at a much greater distance from the lower pole than the intersection of the third with the first.

In the vicinity of the vegetal pole, both first and second cleavage furrows are now only faintly expressed.

In about half the eggs of this stage cell division has proceeded a little more rapidly on one side of the egg than on the other; the cells are smaller in surface view, more numerous, and the cleavage furrows are more uniformly complete (figs. 87, 88 and 210). Thus there is an excentric development of the blastodisc, whereby a condition of bilateral symmetry in the cleavage pattern is produced. This excentric development is a more constant feature in the stages immediately following. The question naturally arises whether this bilateral symmetry in the cleavage pattern has any morphogenetic significance: is it the outward expression of the establishment of the permanent bilateral symmetry and antero-posterior differentiation of the embryo? In other words, does the axis of bilateral symmetry in the cleavage pattern fall in the median plane of the future embryo? This question will be considered in a later paper, in connection with the study of the internal development.

Such an excentric development of the blastodisc has been described for *Amblystoma* and *Necturus* by Eycleshymer ('95 and '04), who cites similar observations on other vertebrates by various writers. Eycleshymer speaks of a "second area of accelerated cell division" as distinguished from the primary area

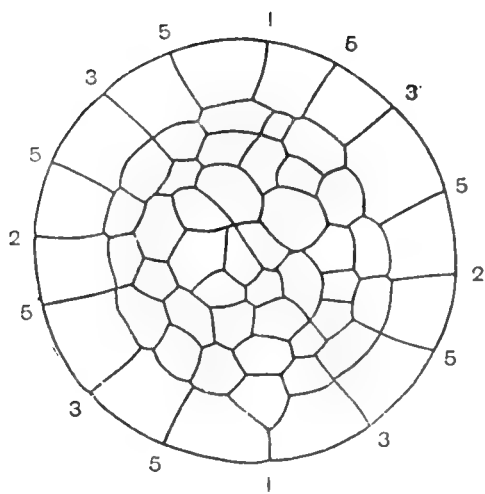
of cell division at the animal pole. In *Cryptobranchus* what happens seems to be a shifting of the most active area of cell division to an excentric position in the blastodisc; hence I have preferred to speak of it merely as a process of excentric development.

No constant relation exists between the axis of bilateral symmetry due to excentric development and the original direction of the first cleavage furrow as shown by those portions of it that have not undergone shifting. The two may coincide (fig. 88); they may be at right angles to one another; they may be oblique (fig. 87).

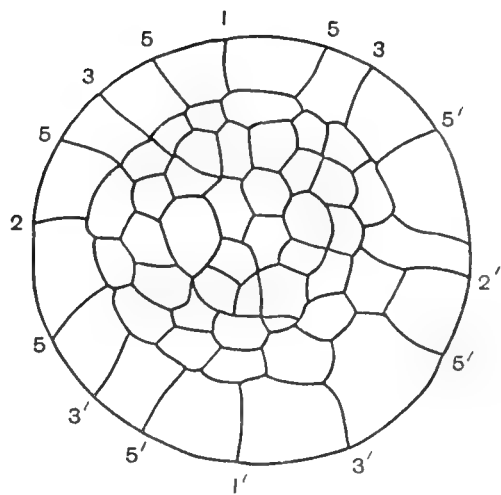
As already indicated, in this stage the cleavage pattern of *Necturus* bears a strong resemblance to that of *Cryptobranchus allegheriensis*, but there is this marked difference: the third cleavage furrows of *Necturus*, when vertical, usually join the first at a greater distance from the vegetal pole, in the region of the equator. In most eggs of *Necturus* examined in this stage only the first two cleavage furrows extend into the lower hemisphere; these usually meet at right angles at the vegetal pole. Thus the cleavage of *Necturus* in this stage seems to show an even stronger tendency toward the meroblastic condition. But this is merely a consequence of the tendency for the third cleavage furrows to come in obliquely or latitudinally; a comparison of later stages shows that the meroblastic tendency is in reality a trifle less strongly expressed in *Necturus* (figs. 107 and 108) than in *Cryptobranchus*.

In *Amia* (Dean '96; Whitman and Eycleshymer '97) the fifth cleavage furrows appear in two sets: an outer set cutting the eight macromeres latitudinally; and an inner set cutting the four micromeres in a horizontal plane, hence not visible from the surface.

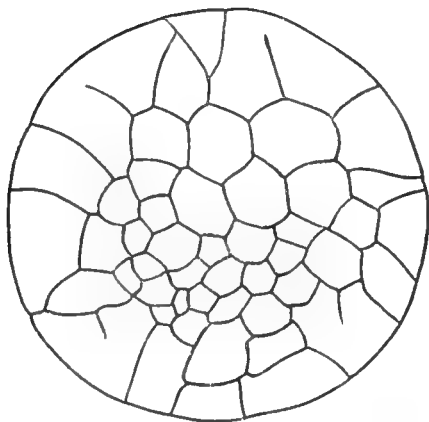
Stage 6: (figs. 97 to 102 and 212 to 214). This stage is characterized by the presence of the sixth cleavage furrow, giving a maximum of sixty-four cells, some of the macromeres being incompletely divided. Considerably more than half the cells are micromeres; these occupy an area whose diameter extends over only about 90° of the circumference of the egg. Hence the mero-



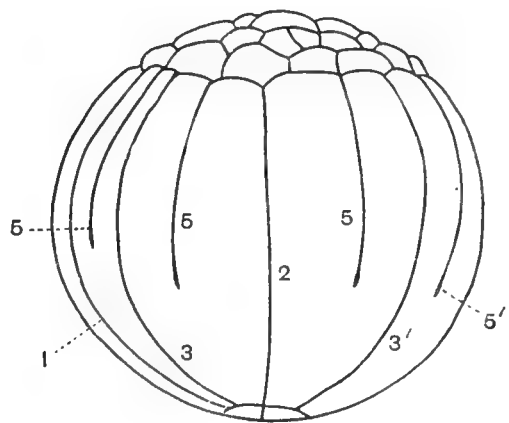
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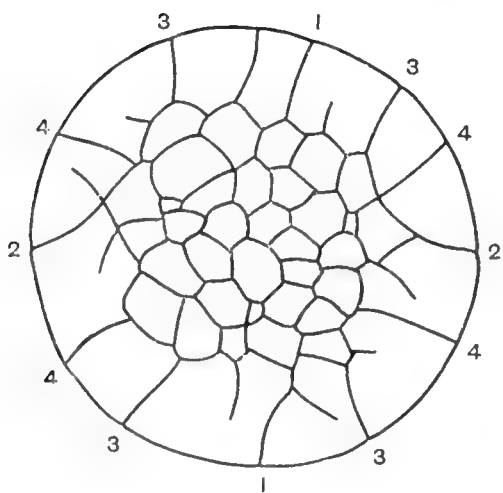
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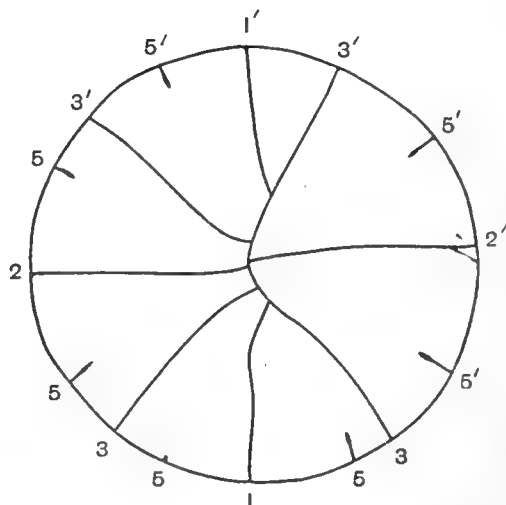
98



101



99



102

Figs. 97 to 102 Sixth cleavage (Stage 6) of *Cryptobranchus allegheniensis*. Figures 100 to 102 show upper hemisphere, equatorial view, and lower hemisphere, respectively, of the same egg. All of the figures are camera drawings from preserved material. Figure 98 is drawn from the egg photographed for figure 212. $\times 7$.

blastic tendency is strongly expressed. This stage is reached about four hours later than the beginning of the preceding stage.

A description of a few individual eggs will best indicate the characteristics of this cleavage.

Out of about fifty eggs studied, the one represented in figure 97 shows the greatest regularity of cleavage in the upper hemisphere. This condition must have been reached by a fairly constant alternation of vertical and latitudinal cleavage furrows. This alternation of cleavage furrows carried out with completeness and geometrical precision would give a total of sixty-four cells, consisting of forty-eight micromeres and sixteen macromeres; the micromeres would be arranged in three concentric rows, each containing sixteen cells. In the egg under consideration, this condition is realized in the outer row of cells, which is quite regular and contains the theoretical number, sixteen. But the total number of micromeres is only thirty-nine, hence a deficiency must exist in the central portion of the blastodisc, or some divisions in this region must have taken place horizontally. Sections show that no horizontal divisions have taken place in this region; on the other hand divisions parallel to the surface have sometimes occurred in the marginal row of micromeres. Therefore cell division is taking place more rapidly in the marginal than in the central region of micromeres—a condition which may be the beginning of that accelerated development of the margin, the later expression of which is almost wholly internal.

A study of other eggs showing a fairly regular alternation of cleavage furrows gives additional evidence for this interpretation (e.g., fig. 100, representing an egg with 39 or 40 micromeres). While eggs with this degree of regularity in the cleavage pattern are the exception rather than the rule, it is felt that evidence derived from them is especially trustworthy; for in such eggs the equilibrium in the rhythmic alternation of the direction of cell division has been best maintained, and the rather uniform lagging-behind of the divisions of the cells in the central area would seem to be the expression of a normal tendency in the life of the embryo. In eggs which show disturbances in this equi-

librium, discordant factors are more likely to be present to obscure the normal expression of the course of development.

The sixth cleavage furrows of the outer set, when latitudinal, divide the macromeres very unequally, cutting off additional micromeres. The number of micromeres, and the extent of the blastodisc, is increased by such latitudinal divisions; the number of macromeres is increased by the sixth cleavage furrows only when these come in vertically.

In a few eggs, as the one shown in figure 99, there is a marked tendency for the sixth cleavage furrows to come in vertically. Here, as noted in an earlier stage, the embryo seems to be oscillating between two possible modes of cleavage; but the tendency to preserve the regular alternation of cleavage furrows is usually the stronger.

The most marked tendency to vary from the regular pattern of cleavage occurs along the line of excentric development of the blastodisc (figs. 98 and 212), as described under Stage 5. The majority of eggs exhibit this tendency in some degree.

We have then, in the cleavage pattern of this stage, two tendencies toward differentiation of the blastodisc: (a) an accelerated cell division in the marginal portion, pointing toward the formation of the germ ring; and (b) an accelerated cell division about a radius of the blastodisc, giving a condition of bilateral symmetry.

DeBussy's figure ('05, fig. 10) representing the blastodisc of an embryo of *Cryptobranchus japonicus* with forty micromeres strongly suggests excentric development; on one side of the first cleavage furrow only three cleavage furrows reach the equator, on the other side nine. But the author remarks (p. 530) that he has observed no secondary center of accelerated cell division such as has been described by Eycleshymer for *Necturus*.

A comparison with earlier stages shows that there is an increasing tendency for the micromeres, following a familiar law of developmental mechanics, to lose their original quadrangular or triangular outline and become hexagonal.

In the living egg, the roof of the segmentation cavity is somewhat translucent, and spaces communicating with the cavity

beneath sometimes appear between the cells. Evidently the roof consists of a single layer of flattened cells; this inference is confirmed by the study of sections.

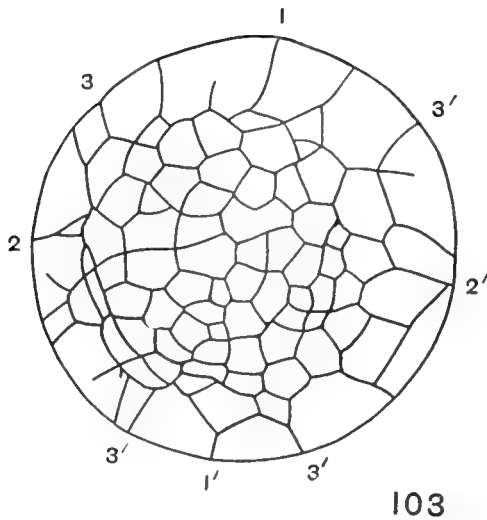
On account of the slow cleavage and relative stability of the macromeres, there is little change in the cleavage pattern of the lower hemisphere. An advance is shown in that the vertical fifth cleavage furrows have invaded the lower hemisphere (fig. 102). Those fourth cleavage furrows that proceed vertically are seldom complete in this stage, but sometimes are found joining an earlier vertical furrow at a considerable distance from the vegetal pole.

In this stage the most regular type of cleavage pattern of *Cryptobranchus* bears a striking resemblance to the corresponding stage of *Amia* (Dean '96; Whitman and Eycleshymer '97).

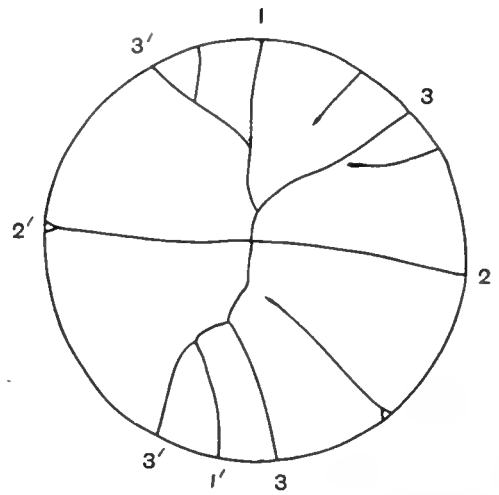
Since Stage 6 of the egg of *Cryptobranchus* best serves to illustrate the fundamental characteristics of the cleavage pattern, particularly with regard to the relative size of the micromeres and macromeres, at this point a comparison may well be made with the dipnoi and crossopterygii. The general anuran or urodele character of the cleavage of the dipnoan egg is apparent in all existing genera: *Ceratodus* (Semon '00 and '01); *Protopterus* (Budgett '01; Kerr '09); and *Lepidosiren* (Kerr '00, '01 and '09). With respect to inequality in the cleavage, *Lepidosiren* in particular closely approaches the condition in *Cryptobranchus* and *Necturus*. The cleavage of *Polypterus* (Kerr '07) bears a general resemblance to that of *Amblystoma* and the frog.

Stage 7: (figs. 103 to 105; 215 and 216). This stage is characterized by a doubling of the number of cells found in the preceding stage, and by a slight extension of the region occupied by the micromeres. The stage is reached about four hours later than Stage 6.

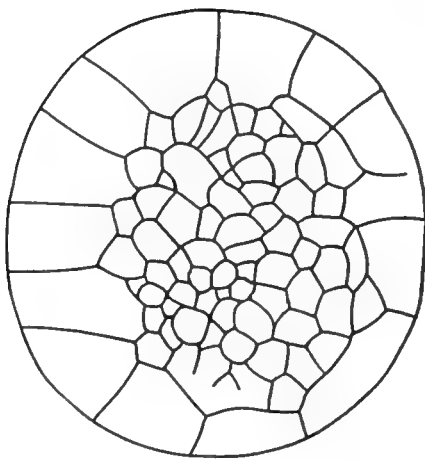
Figures 103 and 104 show a fairly representative egg in this stage. The cells in the region of the animal pole are markedly larger than the other micromeres. This condition may be due to one or both of two factors: (a) the flattening of the cells composing the roof of the segmentation cavity; (b) a slower rate of division in these cells, as noted in Stage 6. There is marked



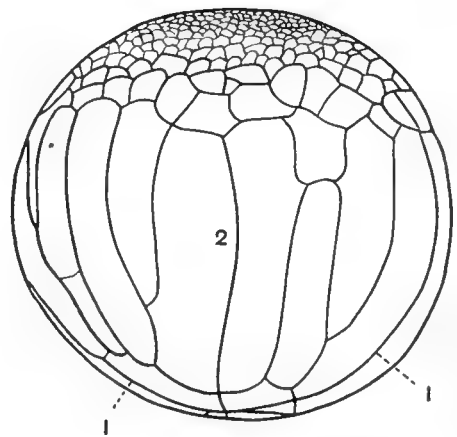
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Figs. 103 to 105 Surface views of eggs of *Cryptobranchus allegheniensis* in Stage 7, showing cleavage furrows. Figures 103 and 104 represent upper and lower hemispheres, respectively, of the same egg. Camera drawings from preserved material. $\times 7$.

Fig. 106 Equatorial view of an egg in Stage 8, showing cleavage pattern. Camera drawing from preserved material. $\times 7$.

activity in cell division in an area excentrically situated, though this is not so apparent in the particular egg under consideration as in some other eggs in the same stage. In the lower hemisphere, the biradial character of the cleavage pattern is preserved and accentuated.

In the egg represented in figure 105 we see the beginning of a process of fundamental importance in the further history of

the embryo—the phenomenon of *immigration of cells from the single-layered roof of the segmentation cavity*. In a surface view, it is evident that some of the cells in the excentrically situated area of most rapid cell division are partially submerged. They are not merely smaller superficially than the other micromeres, but are sunken below the general surface and present the appearance of being crowded inward. Their condition will be further described in a consideration of the internal development; their later history and fate form an important phase of the process of embryo-formation.

In most eggs of this stage, at the margin of the blastodisc oblique furrows (probably fifth cleavage furrows) occasionally cut off cells intermediate in size between micromeres and macromeres. In the lower hemisphere some recent furrows, presumably fifth cleavage furrows, usually extend well toward the vicinity of the vegetal pole. The macromeres are, as a rule, long, narrow and wedge-shaped. On account of the segregation of most of the protoplasm within the region of the blastodisc, all latitudinal divisions of the macromeres are very unequal, cutting off new micromeres instead of increasing the number of macromeres. In the living egg, the roof of the segmentation cavity still appears somewhat translucent, and spaces sometimes occur between these cells; but neither of these conditions is so marked as in the preceding stage.

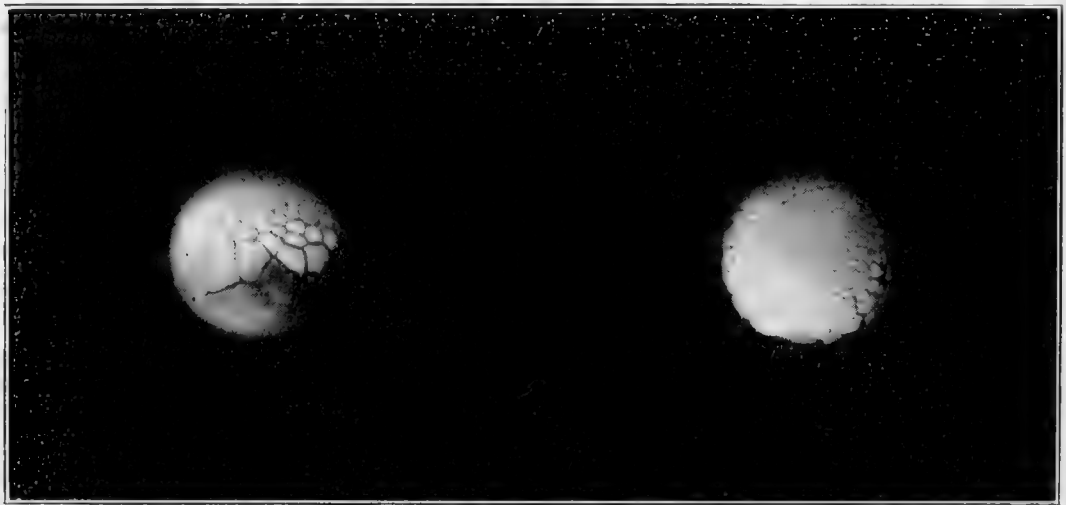
Stage 8: (figs. 106 and 217). This stage is reached about twelve hours later than Stage 7; it is best described by reference to the figures. The micromeres have become much more numerous and smaller; there is a slight extension of their area. There is a more gradual transition, or gradation in the size of the cells, between micromeres and macromeres. In the lower hemisphere the fifth cleavage furrows have, as a rule, become complete; they rarely reach the lower pole, but join an earlier furrow at some distance from the vegetal pole. Biradiality of the cleavage pattern still enables one, as a rule, to distinguish first and second cleavage furrows in this hemisphere.

In the upper hemisphere the excentric area of accelerated cell division noted in the preceding stages is usually quite marked.

In preserved material the nuclei of the micromeres are often easily distinguishable in surface views.

In the living egg, the roof of the segmentation cavity has become quite opaque, and the cells are compactly arranged. During the latter part of this stage a translucent condition begins to appear at the animal pole, indicating a thinning-out of the cells in this region, as in Stage 6; but this time the cells form a firm tissue, with no spaces between them.

In *Necturus* the cleavage furrows in the region of the vegetal pole are fainter than in *Cryptobranchus*; this condition is reversed



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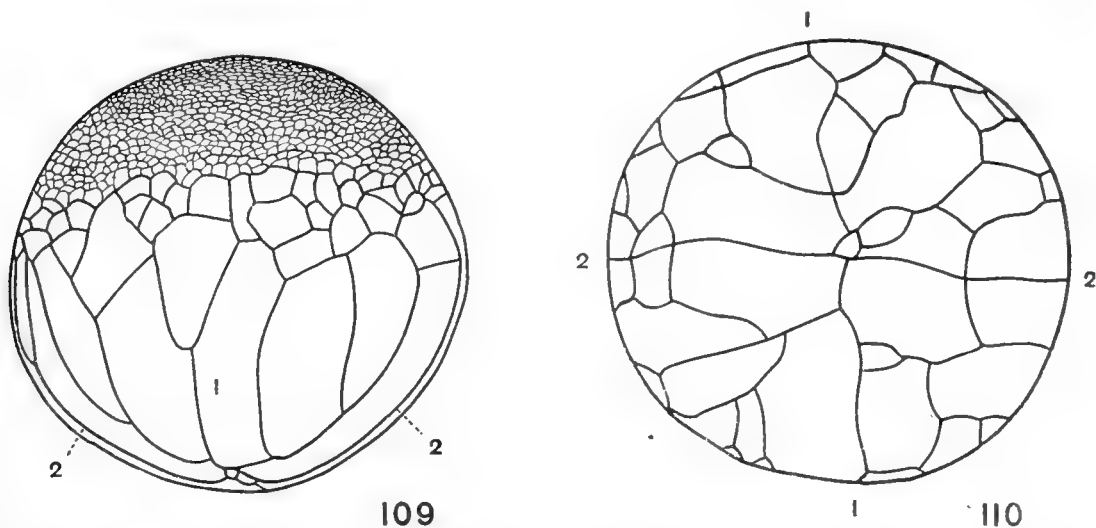
Figs. 107 and 108 Advanced cleavage stage of *Necturus maculosus*. Two views of a single egg, photographed after preservation. $\times 4$.

in the upper hemisphere, where the micromeres are outlined far more boldly in *Necturus* (figs. 107 and 108) than in *Cryptobranchus* (both statements refer to preserved material, fixed by the same method).

Stage 9: (figs. 109, 110 and 218). This stage is reached about nineteen hours later than the preceding stage. Individual micromeres in the region of the animal pole are barely visible to the naked eye. The zone of transition between micromeres and macromeres has become broader and more marked. An eccentrically situated area of accelerated cell division in the micromeres is only occasionally found in surface views of this stage.

In the living egg, the roof of the segmentation cavity appears as a translucent tissue throughout a circular area about 40 degrees in diameter in the region of the animal pole. This indicates a decided thinning-out of the cells of this region.

Biradiality of the cleavage pattern of the lower hemisphere still enables one to distinguish in many embryos, though not in all, the first and second cleavage furrows. Usually two or three cells quite small in surface view occur at the vegetal pole; they are quite characteristic of this and the following stage, but are sometimes found in the preceding stage. At the vegetal

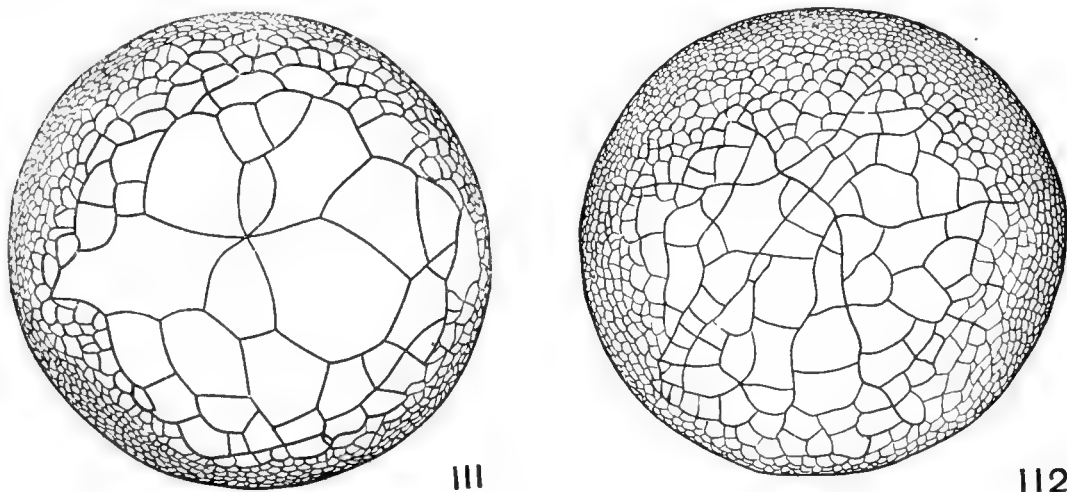


Figs. 109 and 110 Stage 9 of *Cryptobranchus allegheniensis*. Equatorial view and lower hemisphere of different eggs, showing cleavage pattern. Camera drawings from preserved material. $\times 7$.

pole the cleavage furrows, both in living and preserved material, are sometimes both broad and deep, forming quite noticeable fissures; a similar condition is common in *Amblystoma* (Eycleshymer '95). In *Cryptobranchus* this condition is in marked contrast to the stage immediately preceding, in which the furrows in this region were faint. In *Necturus*, on account of the variability of the third cleavage furrows, the biradial pattern of the macromeres is not so clearly expressed as in the egg of *Cryptobranchus*.

Stage 10: (figs. 111, 112 and 219). This stage, reached a day or two later than Stage 9, immediately precedes the beginning of gastrulation. The micromeres at the upper pole are invisible

to the naked eye, and barely distinguishable with the magnification used for photographing ($\times 4$). The area occupied by the micromeres extends approximately to the equator, though the broad zone of transition makes it difficult to define. In the vicinity of the vegetal pole, the cleavage furrows have again become faint; in many cases, in preserved material, they are distinguishable as fine lines lighter in color than the general surface, rather than as actual grooves. For the accurate study of these furrows in this and the following stage, a binocular microscope is usually required. When their boundaries are distinct, on account of their large size the macromeres are readily seen with the naked eye.



Figs. 111 and 112 Lower hemispheres of two eggs of *Cryptobranchus alleghe-niensis* in Stage 10, showing cleavage pattern. The embryo shown in figure 112 is slightly older than the one represented in figure 111. Camera drawings from preserved material. In each egg, the lower pole as determined by gravity lies at the center of the figure; the vegetal pole, at the intersection of the first two cleavage furrows, is slightly above this point. The upper part of each figure represents the side on which the blastopore is to appear. $\times 7$.

Usually, the cleavage pattern of the lower hemisphere retains enough of its earlier bilateral symmetry to enable one to distinguish first and second cleavage furrows. The vegetal pole, since it occurs at the intersection of the first two cleavage furrows, may in most cases still be determined quite accurately and conveniently by means of the cleavage pattern. As shown in figures 111 and 112, the vegetal pole is excentrically located in the area occupied by the macromeres; a more rapid multiplication of cells has occurred on one side of this area, so that on this side

the micromeres and transitional cells approach nearer the vegetal pole. A meridian drawn through the vegetal pole and the center of the area occupied by the macromeres defines the axis of excentricity; this axis bears no constant relation to the first cleavage furrow and the axes of biradial symmetry determined by the early cleavage furrows. The biradial symmetry of the cleavage pattern is of course somewhat disguised by the more rapid multiplication of cells at one end of the axis of excentricity.

In this stage occurs a slight tilting of the morphological axis of the egg within a meridional plane determined by the axis of excentricity, so that the vegetal pole no longer coincides with the lower pole as determined by gravity. The vegetal pole is slightly uptilted on the side where the more rapid multiplication of cells occurs, hence the meridian defining the axis of excentricity passes also through the new pole determined by gravity. This new pole at first lies intermediate between the vegetal pole and the center of the area occupied by the macromeres; in later stages, through continued tilting of the egg in the same direction, it comes to lie beyond this center. Throughout the ensuing stages we must distinguish between the morphological axis of the egg as determined by the animal and vegetal poles, and the vertical axis determined by gravity. The method of locating the vertical axis, and exact measurement of the amount of rotation, will be given in the following stage.

If the egg be sectioned along the axis of excentricity the internal structure, to be described later, shows that this axis lies in the sagittal plane of the embryo; the side on which the small cells approach nearer to the vegetal pole is the one on which the blastopore is to appear. Thus the excentric position of the vegetal pole within the area occupied by the macromeres enables one to orient the egg with reference to future body regions.

In perhaps the majority of cases, the transition from large to small cells is more evenly graded on the side where it occurs nearest to the vegetal pole, than on the opposite side where it is characterized by a rather abrupt line of demarcation (figs. 111 and 112). This feature, when present, gives a true bilateral symmetry to the cleavage pattern of the lower hemisphere; the axis of this bilateral symmetry coincides with the axis of excentricity.

tricity previously defined, hence lies in the sagittal plane of the embryo. The excentric position of the vegetal pole within the area occupied by the macromeres, and the bilateral character of the cleavage in this region, are more marked in many eggs taken immediately after the beginning of gastrulation; these features are usually better expressed than in the eggs shown in figures 111 and 112, which were chosen because the distinctness of the early cleavage furrows enabled them to be drawn with the camera lucida. Schultze ('00, Taf. 11, fig. 12) has described a similar bilaterality in the late cleavage of the lower hemisphere of the frog's egg.

The question of the possible relation of the excentric and bilateral development of the lower hemisphere just described, to the excentric development of the blastodisc noted in previous stages, will be discussed in a later paper.

In the living egg, the roof of the segmentation cavity, though apparently thin, is not quite so translucent as in the preceding stage. It is, however, noticeably more translucent on the side toward the future blastopore, and on this side the transition to the opaque yolk cells is more abrupt.

During the late stages of cleavage, a tendency toward fading out of the cleavage furrows in the vicinity of the vegetal pole has been noted. In some individual cases this process has gone so far that the earlier cleavage furrows are lost to view, even when searched for with the binocular microscope. This tendency may be interpreted as due to a difficulty in sustaining the holoblastic method of cleavage in an egg so heavily laden with yolk. In the corresponding stages of *Necturus*, this tendency is even more marked. My study of the cleavage pattern of the lower hemisphere of the late segmentation stages of both *Cryptobranchus* and *Necturus* has been confined to preserved material, but Professor Dean informs me that he has noticed this merging of lower blastomeres in the late segmentation stages of the living eggs of *Necturus*. My *Necturus* material is not so favorable as the egg of *Cryptobranchus* for the study of the cleavage pattern in this stage, so a detailed comparison will not be attempted.

In the very late cleavage stages of *Cryptobranchus japonicus*, Ishikawa ('08 and '09) describes a shallow furrow ('Scheidewand-

furche' or 'septal furrow') bounding the posterior margin of the roof of the segmentation cavity, parallel to the future blastopore. Such a furrow does not normally occur in this stage of *Cryptobranchus allegheniensis*, but a similar furrow makes its appearance shortly after the beginning of gastrulation.

B. Summary

The cleavage is holoblastic, but with great inequality in the size of the micromeres as compared with the macromeres.

Biradial symmetry of the cleavage pattern begins with the third cleavage stage. In the upper hemisphere, as a consequence of the shifting of the micromeres, this biradial symmetry is lost at about the fifth cleavage stage. In the lower hemisphere, because of the slow cleavage and stability of the macromeres, it persists until after the beginning of gastrulation, and in some eggs enables one to distinguish first and second cleavage furrows even after the blastopore has appeared.

An excentrically situated area of unusually small micromeres is apparent in surface views of most eggs in Stages 5 to 8 inclusive; the cleavage pattern of such eggs thus possesses bilateral symmetry.

In the sixth cleavage stage there is more rapid cell division in the marginal region of the micromeres than in the region of the animal pole. The later expression of this tendency is almost wholly internal.

In Stages 7 and 8 some of the superficially smaller micromeres are becoming submerged through a process of immigration.

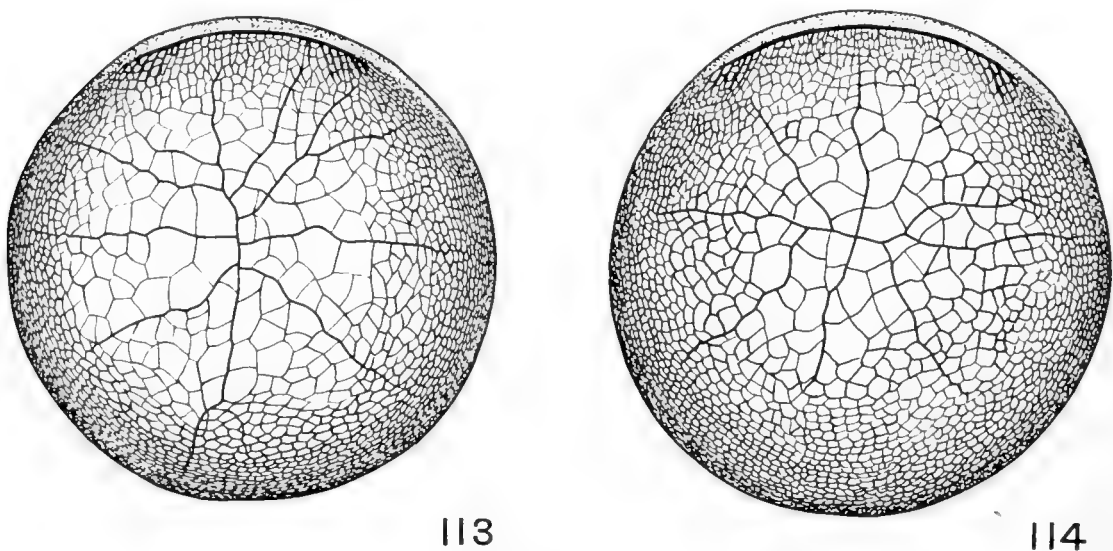
In the later cleavage stages there is a tendency for the cleavage furrows to become less distinct than formerly in the region of the vegetal pole, indicating a difficulty in sustaining the holoblastic character of the cleavage in an egg so heavily laden with yolk; the same tendency is observed in *Necturus*.

In late segmentation immediately preceding gastrulation the cleavage pattern enables one to predict the side on which the blastopore is to appear; the egg undergoes a slight rotation on a horizontal axis at right angles to the median plane.

VIII. GASTRULATION AND EARLY FORMATION OF THE EMBRYO

A. Description by stages

Stage 11: (figs. 113 to 137 and 220 to 222). This stage extends from the time of the first appearance of the blastopore as a short horizontal groove until its ends meet to form a complete circle. In eggs kept in their natural environment, gastrulation begins about seven days after fertilization and two days after the beginning of Stage 10.



Figs. 113 and 114 Lower hemispheres of two eggs of *Cryptobranchus allegheniensis* in an early gastrula stage, showing cleavage furrows. The vertical axis as determined by gravity lies at the center of each figure; the vegetal pole, at the intersection of the first two cleavage furrows, is about 7 degrees above the vertical pole. In figure 113 the first cleavage furrow lies approximately in the median plane of the gastrula; in figure 114 it is at right angles to this plane. Camera drawings, finished under the binocular, from preserved material. $\times 8$.

The blastopore is first distinguished as a shallow irregular and broken horizontal groove two or three millimeters in length, lying about 15 degrees below the equator. It occurs at the upper limit of transitional cells between micromeres and macromeres, and its immediate site is distinguished by a rather abrupt demarcation between micromeres and distinctly larger transitional cells. The groove is started, not by a lining-up of cells and the union of cleavage furrows, as described by Eycleshymer ('95) for *Amblystoma*, but by the sinking-in of groups of entire cells

at intervals along a narrow zone several cells in width; hence from its very beginning the process is not a splitting-apart of cells, but invagination. The groove soon becomes continuous and deepens by the inturning of cells along both margins.

After the groove has reached a length of three millimeters or more, the process of invagination becomes accompanied by one of overgrowth or epiboly: the dorsal lip grows slowly down over

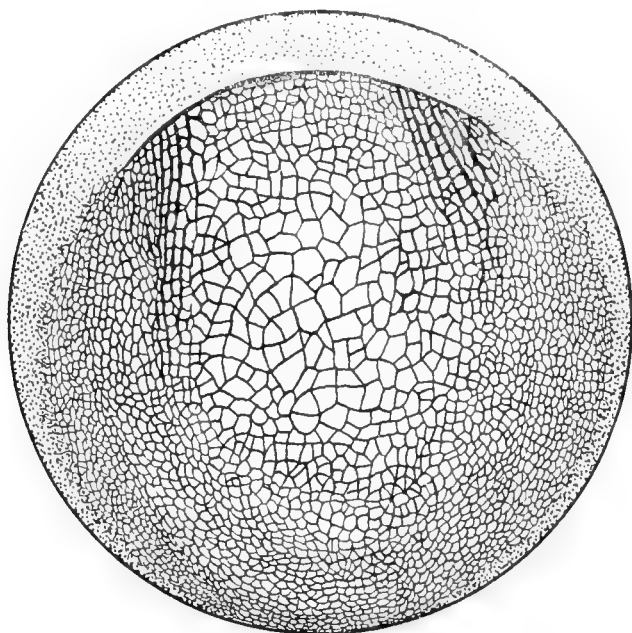


Fig. 115 Lower hemisphere of a gastrula of *Cryptobranchus alleghehiensis*, in a slightly later stage than the preceding, showing the lining-up of the cells within the horns of the blastopore. Freehand drawing from a photograph of preserved material. $\times 10$.

the cells transitional between micromeres and macromeres (figs. 113 to 115). As shown in figure 115, the transitional cells just within the horns of the blastopore are elongated as if compressed; here the cells line up and lengthen out at right angles to a line connecting the horns of the blastopore.

After the blastoporic groove has attained the form of a semi-circle (fig. 133), a zone of rather abrupt demarcation between micromeres and transitional cells completes the circle begun by the crescentic blastopore; this zone marks the site of the ventral lip of the blastopore. A little later, the blastoporic groove extends rapidly along this line of demarcation, becoming an almost

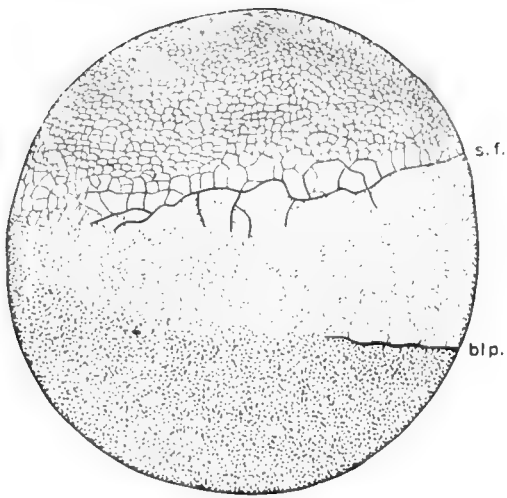
perfect circle, and enclosing a broad horseshoe-shaped band of transitional cells, within which lie the macromeres (fig. 138).

It has already been noted that a slight rotation of the egg on a horizontal axis has taken place in Stage 10, so that it is now necessary to distinguish between the morphological axis of the egg and the vertical axis determined by gravity, since the two no longer coincide. In the study of gastrulation this rotation must be taken into account, and some means must be found for measuring it. In studying the morphological features of the egg (position of blastopore, etc.) in their relation to the vertical axis, two general methods have been used: (a) the living egg, placed in a small vial of water, has been studied in side view and measurements made against a protractor used as a background; and (b) for accurately locating the vertical axis I have devised the following apparatus: a glass disc such as is used for an ocular micrometer was marked in the center with a small dot; a circle with a radius of 4 mm. was then drawn about this dot as a center. When this disc is placed in the eyepiece of a low-power microscope used in studying the eggs, the circle is just large enough to enclose the image of an egg. When the egg, immersed in water in a watch glass, is accurately placed so that its image is enclosed by the circle, the dot lies over the upper pole of the vertical axis; this point is then marked by puncturing with a hot needle. The operation was first tried on living eggs, which were then fixed for further study; but since with the living egg even a small puncture in this region usually causes the embryo to collapse during the subsequent process of fixation, in general this method is less satisfactory with living than with preserved material. On account of the usually perfect preservation of the form of the egg by the fixing fluid employed, the results obtained by marking preserved material seem fairly trustworthy, especially when spherical eggs are selected and a large number used. The position of the upper vertical pole, thus marked, gives a reference point for correlating the morphological features of the egg with the vertical axis; the measurements were made by means of camera drawings.

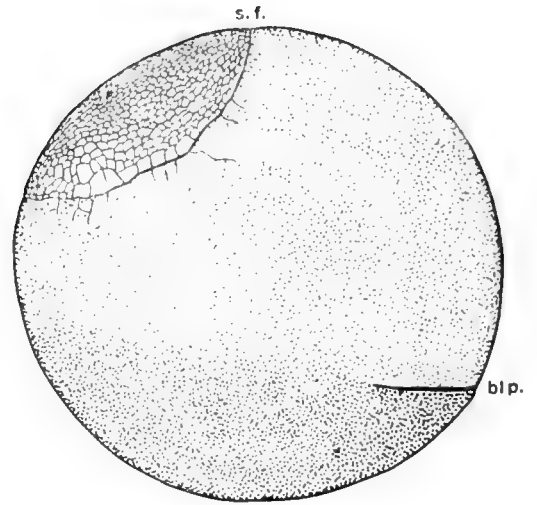
In making these measurements, it is especially necessary to guard against using eggs with an unusually large yolk plug, since this is one of the commonest abnormalities. Moreover, even in perfectly normal eggs there is considerable variation in the position of the blastopore, so that a large number of eggs must be studied and averages taken. The results obtained by the two methods agree closely.

Since the blastopore, at the time of its first appearance is only about 15 degrees below the horizontal equator and approximately parallel to it, the blastopore at first forms an arc of an imaginary circle whose diameter, measured along a meridian of the egg, is about 150 degrees. At the time when the blastopore has reached the form of a semicircle, this diameter measures about 125 degrees; when the blastopore has become a complete circle the average diameter, in normal embryos, is only 94 degrees. Therefore the crescentic blastopore forms an arc of a circle of steadily diminishing diameter; the lips of the blastopore, and the entire germ ring (to be described in a later paper), contract as they progress slowly downward over the egg.

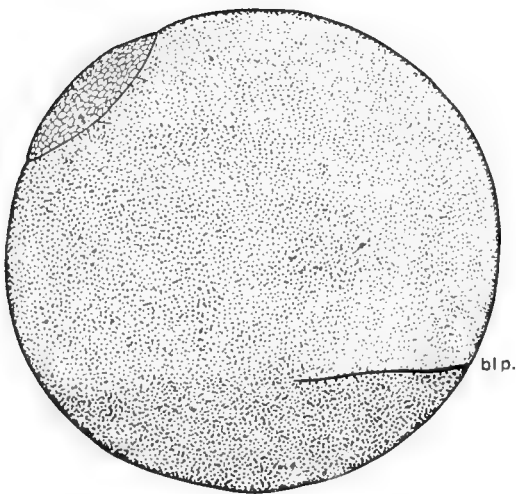
In preserved material the cleavage pattern of the macromeres is still fairly well defined; by means of careful study with a binocular microscope it is usually possible to distinguish first and second cleavage furrows (figs. 113 and 114). This enables a direct comparison to be made between the direction of the first cleavage furrow and the median plane of the gastrula; this point will be discussed in a later paper. The identification of early cleavage furrows in this region is furthermore of importance in enabling one to determine the position of the vegetal pole, since this is located at the intersection of the first two cleavage furrows. Measurements show that at the time when the blastopore is first clearly established, the vegetal pole lies, on the average, 68 degrees below it, and 7 degrees above the lower pole of the vertical axis. At the time when the blastopore becomes a semicircle, the vegetal pole lies only 32 degrees below its dorsal lip; when the blastopore first becomes a complete circle the vegetal pole lies only 26 degrees below the dorsal margin of the yolk plug. During this time continued rotation of the egg has brought its



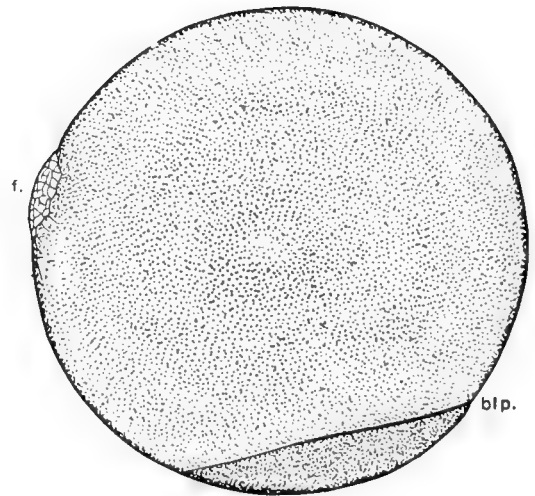
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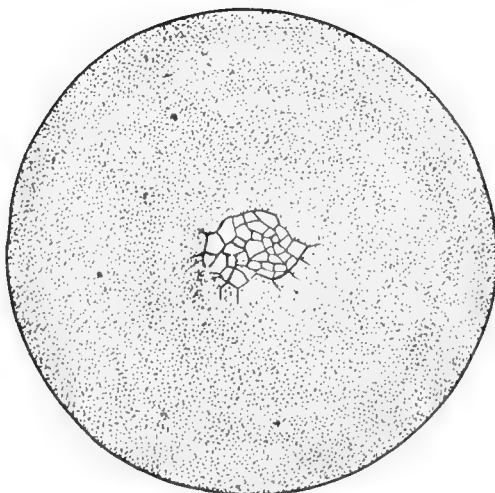
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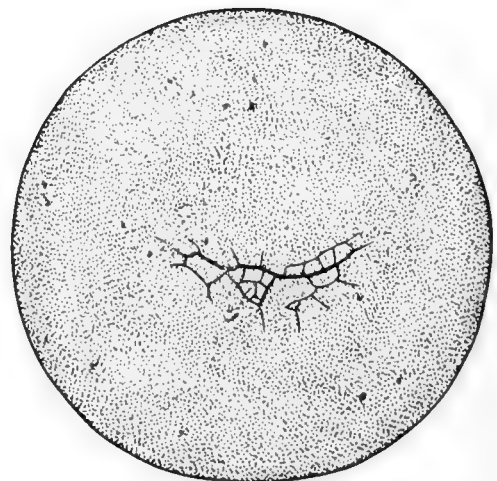
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morphological axis to an angle of 44 degrees from the vertical; the ventral lip of the blastopore now lies about 24 degrees beyond the lower pole of the vertical axis. These changes are set forth diagrammatically in figures 134 to 137.

Two quantitative results of considerable importance are brought to light through the study of these data: (a) the dorsal lip of the blastopore has grown downward over the yolk cells for a distance of about 42 degrees; (b) the egg has rotated in the opposite direction about 37 degrees from its position at the beginning of gastrulation, making a total rotation of 44 degrees. At first, overgrowth is more rapid than rotation; at the time when the blastopore has reached the form of a semicircle its dorsal lip is 43 degrees below the horizontal equator. Later, rotation is more rapid than overgrowth, and at the time when the blastopore has become a complete circle its dorsal lip has been carried back to a position 20 degrees below the horizontal equator, only 5 degrees lower than its original position in space.

The changes in the upper hemisphere visible from the surface during the establishment of the blastopore are remarkable, since they afford clues to many important processes within. In the living egg especially, because of the translucent character of the upper hemisphere, one is able to get total views of many phases of gastrulation, such as could not be obtained from serial sections except by means of reconstructions. Except where otherwise noted, the following description is based on the study of the living egg.

At the very beginning of the process of gastrulation, the nearly transparent roof of the segmentation cavity is of quite uniform

Figs. 116 to 121 Stage 11 (gastrula) of *Cryptobranchus allegheniensis*. Camera drawings from preserved material. In all the figures, the upper vertical pole as determined by gravity lies toward the top of the page; *blp.*, dorsal lip of the blastopore; *f.*, fenestra (roof of the blastocoele differentiated into a window-like structure); *s.f.*, septal furrow. $\times 7\frac{1}{2}$.

Fig. 116 Lateral view of an early gastrula stage. The sharp differentiation of the fenestra is rather precocious in this egg.

Figs. 117 to 119 Lateral views of a characteristic series of later embryos.

Figs. 120 and 121 Antero-ventral views showing stages in the disappearance of the fenestra. Figure 120 is from the egg drawn for figure 119.

extent about the animal pole as a center, covering an area about 140 degrees in diameter. As gastrulation advances this clear area becomes encroached upon at its posterior margin (figs. 122 and 123) by the extension of the opaque material. Meanwhile the boundary of the roof of the blastocoele becomes more sharply defined; before the upgrowth of the postero-dorsal opaque region has reached the animal pole the margin of the blastocoele roof is usually bounded by a sharply defined furrow, the 'septal furrow' of Ishikawa (see below)—a characteristic and almost unique feature of the gastrulation of *Cryptobranchus*. The precise stage at which this furrow appears varies considerably in different eggs; figure 116 shows a case of unusually early appearance, figures 117, 125 and 126 a stage in which it is usually well established. Moreover, the distinctness of this groove varies greatly, particularly in eggs of different spawnings; in some lots of eggs the groove is established early and is very sharply marked, while in occasional lots of eggs it is almost absent.

The septal furrow appears first at the posterior margin of the roof of the segmentation cavity, then extends gradually around to its anterior margin; in its appearance and manner of extension

Figs. 122 to 133 Stage 11 (gastrula) of *Cryptobranchus allegheniensis*. Free-hand drawings of the living eggs, viewed by both transmitted and reflected light; the proportions of the various parts are checked by comparison with camera drawings of preserved material. The drawings are oriented with respect to the vertical axis determined by gravity. The roof of the segmentation cavity is nearly transparent; the roof of the gastrocoele is quite translucent, or slightly opaque in the regions containing mesoderm; heavily yolk-laden regions are decidedly opaque; *bc.*, roof of blastocoele; *blp.*, dorsal lip of the blastopore; *f.*, fenestra (roof of the blastocoele differentiated into a roof-like structure); *gc.*, gastrocoele; *m.*, region containing mesoderm. $\times 5$.

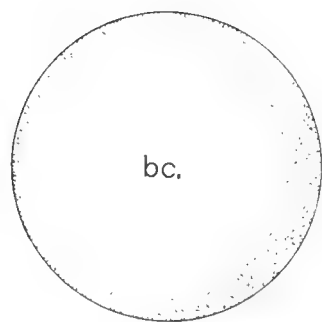
Figs. 122 to 124 Upper hemisphere, lateral view and lower hemisphere of an egg in the beginning gastrula stage.

Figs. 125 and 126 Upper hemisphere and lateral view of an egg a little later than the preceding.

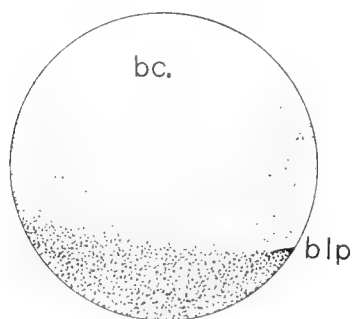
Figs. 127 to 129 Postero-dorsal view, upper hemisphere and lateral view of an egg slightly later than the preceding.

Fig. 130 Upper hemisphere of a slightly later egg.

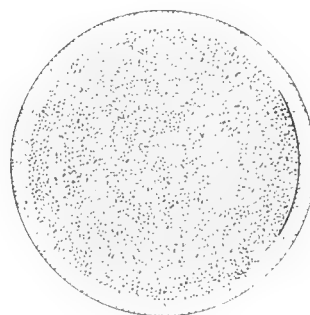
Figs. 130 to 133 Upper hemisphere, postero-dorsal view and lower hemisphere of an egg near the close of Stage 11 (shortly before the appearance of the neural groove).



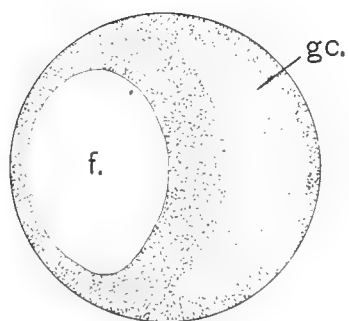
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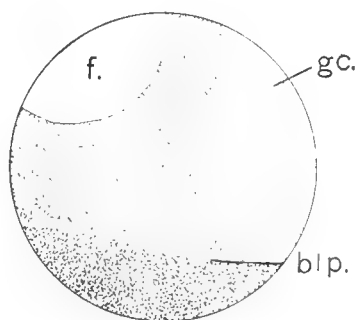
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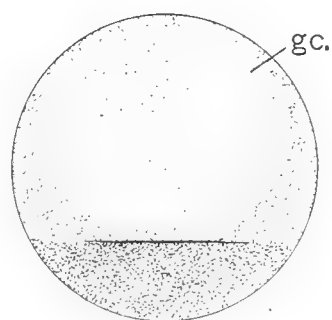
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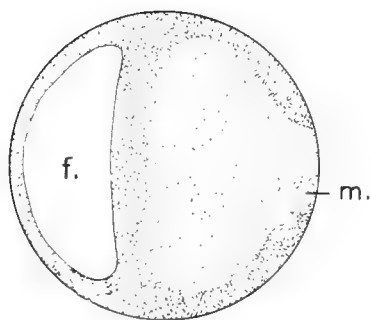
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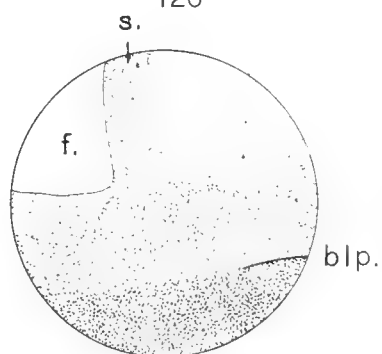
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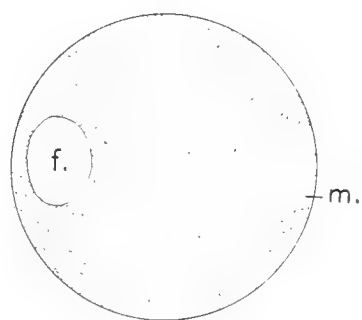
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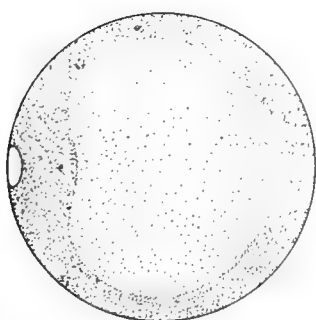
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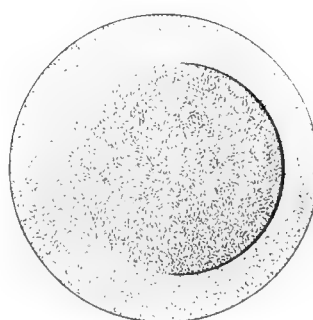
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133

it somewhat resembles a blastopore (fig. 221). Later, the groove becomes faint at its posterior margin, very pronounced at its antero-ventral margin (fig. 222). The area enclosed by the groove diminishes in size with its forward movement; it also becomes almost transparent. Since throughout the remainder of its history this area strikingly resembles a window, I shall refer to it as the *fenestra*.

In material fixed in a modification of the bichromate-acetic-formalin mixture (see Smith '12, Section III, Solution B) containing twice the usual amount of potassium bichromate, the fenestra is cut up into small polygonal areas separated by furrows that greatly resemble cleavage furrows (figs. 116 to 120 and 222; cf. Ishikawa, '08 and '09). These polygonal areas do not represent single cells; each comprises a group of several cells. The phenomenon is not entirely an artifact, since it often appears, though faintly, in the living egg. By this method of fixation the septal furrow is likewise accentuated.

Before describing the further history of the fenestra it is desirable to direct attention to some other changes in the upper hemisphere as observed in the living egg.

About the time that the fenestra becomes limited to the anterior half of the upper hemisphere by the upgrowth of the posterior margin of the opaque region, a translucent area, the roof of the gastrocoele, appears in this region of upgrowth (figs. 125 and 126). This translucent area is at first crescent-shaped; it is separated from the more transparent fenestra by an opaque band which is the outward expression of the septum separating the gastrocoele from the blastocoele.

As soon as the septum has advanced into the anterior half of the upper hemisphere, the translucency of the roof of the gastrocoele extends backward almost to the blastopore—evidently by the deepening of the gastrocoele in this region, admitting light. Meanwhile each postero-lateral margin of this region becomes bordered with a faint band of a slightly more opaque character—an effect due largely to the early mesoderm (figs. 127 to 129), though the entoderm is also concerned in producing it.

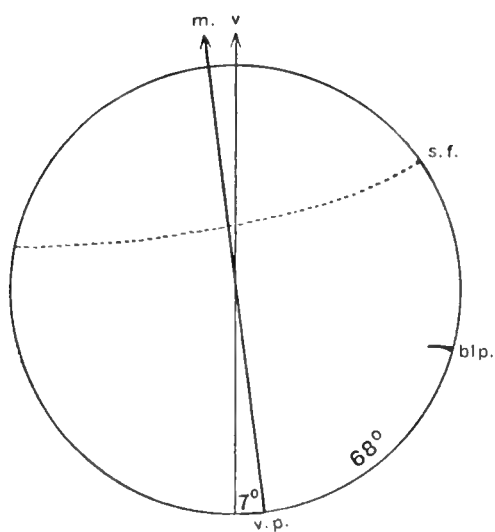
Later changes are concerned with the forward, or rather ventrad, progress of the septum and the increase in the extent of the translucent roof of the gastrocoele, with a correlated ventrad movement of the fenestra and a diminution of its area; there is a slight increase in the extent and opacity of the mesoderm (figs. 118 to 120 and 130 to 132). The fenestra finally closes just below the horizontal equator on the ventral side of the egg (fig. 121). The changes in the position and extent of the fenestra are shown diagrammatically in figures 134 to 137.

The foregoing detailed account of the progress of the septum as viewed from the exterior in the living egg of *Cryptobranchus* clears up whatever doubt may exist as to the significance of the 'shadowy area' described in the gastrula of *Spelerpes* by Goodale ('11) and confirms his suggestion as to the nature of this area.

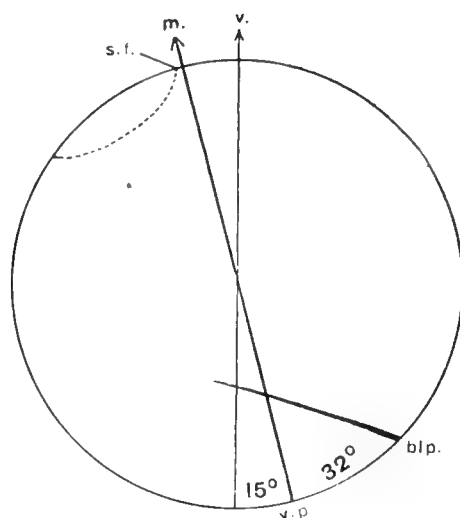
Ishikawa ('08 and '09) describes in the early gastrula of *Cryptobranchus japonicus* a furrow bounding the roof of the blastocoele at its posterior margin, which he calls the 'Scheidewand-furche' or 'septal furrow.' As compared with the furrow of similar nature described above for *C. allegheniensis*, it is earlier in making its appearance, since it antedates the blastopore. The area later enclosed by this furrow has been named by Ishikawa the 'Keimhohlensegment' or 'blastocoele-segment'; judging from his figures its later history is much the same as that of the corresponding structure, which I have preferred to call the 'fenestra,' in *Cryptobranchus allegheniensis*.

The only mention of similar structures which I can find in the literature on other forms is a description by Hatta ('07) of a groove which he calls the 'boundary groove' in the gastrula of *Petromyzon*. As compared with the septal furrow of *Cryptobranchus* this groove is greatly exaggerated in *Petromyzon*, constricting the egg so that in some cases it assumes an hour-glass form.

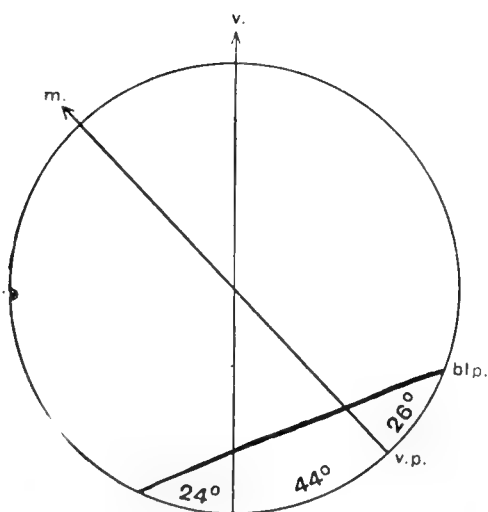
As suggested by Hatta, the boundary groove or septal furrow is passive in origin, and a product of gastrulation. Similar conditions have produced it in two such widely separated forms as *Cryptobranchus* and *Petromyzon*; in each case the egg contains considerable yolk, and the roof of the blastocoele is unusually



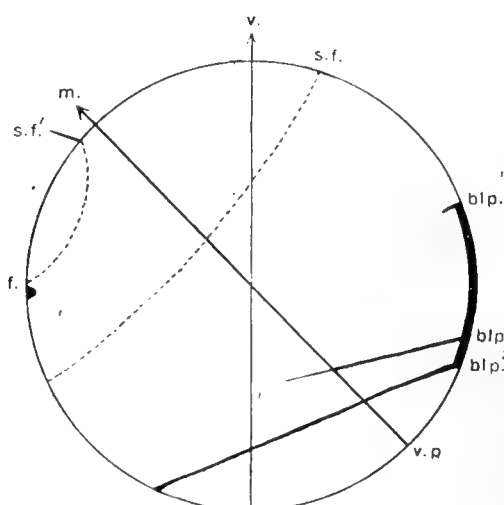
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Fig. 134 Diagram of an egg of *Cryptobranchus allegheniensis* in the beginning gastrula stage viewed from the lateral aspect, showing average amount of rotation, and the positions of the beginning blastopore and the septal furrow; *blp.*, blastopore; *m*, morphological axis; *s.f.*, septal furrow; *v*, vertical axis determined by gravity; *v.p.*, vegetal pole.

Fig. 135 Similar diagram of a gastrula at the time when the blastopore reaches the form of a semicircle. Lettering as before.

Fig. 136 Similar diagram of a gastrula at the time when the blastopore first becomes a complete circle. Lettering as before.

Fig. 137 Combination of the preceding diagrams. The egg is shown in the position assumed at the close of the period considered. The black band indicates the amount of overgrowth of the dorsal lip of the blastopore (42 degrees); *blp.*, *blp'*, and *blp²*, mark the successive positions of the dorsal lip of the blastopore; *s.f.* and *s.f'*, successive positions of the septal furrow; *f*, position of the vanishing fenestra. Other lettering as in the preceding figures.

thin. As stated by Ishikawa, the polygonal figures formed on the surface of the blastocoele-segment (fenestra) are perhaps due to the pressure which produces the gradual diminution of its area; but the cells of the fenestra are not compacted together to any considerable extent, since the gastrocoele roof and wall merely grow under them.

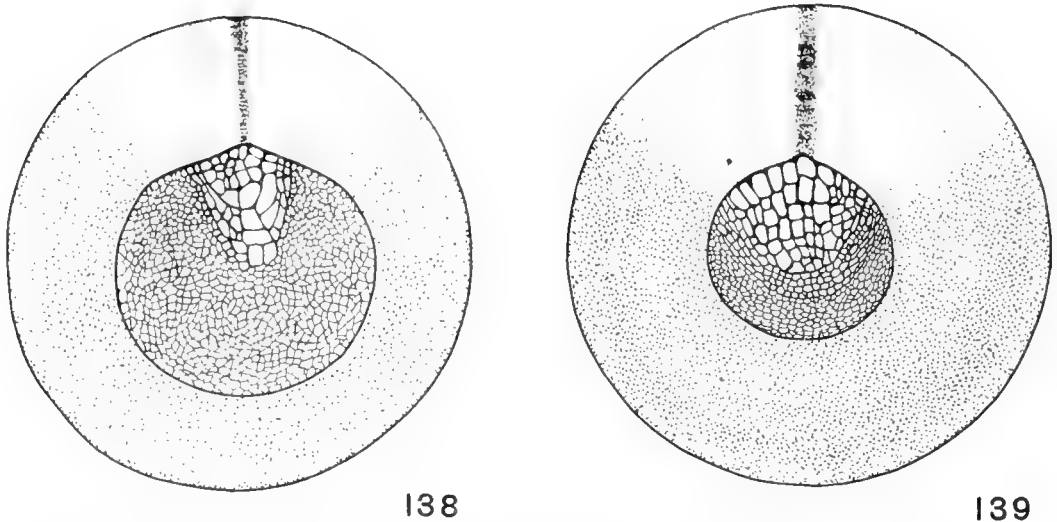
In view of the later history, it is evident even from surface views that in the stage shown in figures 116 and 123 the formative material for the embryo is mainly concentrated in the equatorial region as a broad band or zone of cells, wider in its posterior portion. As will be shown in the description of the internal structure, this equatorial zone as distinguished in surface views is only roughly comparable to the germ ring of fishes.

My material is lacking for the study of the early gastrula stages of *Necturus*; late gastrula stages differ from *Cryptobranchus* chiefly in that the blastopore earlier becomes a complete circle. In *Spelerpes*, according to Goodale ('11), no ventral lip is formed to the blastopore. As compared with urodele and anuran eggs in general, the blastopore of *Cryptobranchus* is late in closing; in its mode of gastrulation the egg of *Cryptobranchus* approaches more nearly the type observed in meroblastic eggs.

Stage 12: (figs. 138 to 150 and 223 to 225). This stage is characterized by the presence of the neural groove and is terminated by the appearance of the neural folds. The neural groove appears about three days after the beginning of gastrulation.

At the time of the earliest indications of the neural groove, the blastopore has just become a complete circle. At the close of the preceding stage it had a diameter of about 94 degrees; it now rapidly becomes smaller, so that before the appearance of the neural folds its diameter averages about 26 degrees (figs. 138 to 145).

During the early part of this stage the yolk plug is characterized by a broad crescent-shaped or horseshoe-shaped area of smaller cells lying ventrad and laterad to the macromeres (figs. 138 and 139). Along the lateral line of transition between the macromeres and these smaller cells, the cells appear compressed and exhibit a tendency to line up and merge their cleavage furrows (figs.



Figs. 138 and 139 Posterior views of embryos of *Cryptobranchus allegheniensis* in Stage 12, showing the condition of the blastopore and the cleavage furrows of the yolk plug. Camera drawings from preserved material. The embryos are not accurately oriented with respect to the vertical axis determined by gravity. $\times 7$.

Fig. 138 Showing condition shortly after the appearance of the neural groove.

Fig. 139 A little later than the preceding.

138 to 142; cf. figs. 113 to 115). Toward the close of this stage, the smaller cells become completely overgrown by the ventral and lateral lips of the blastopore, leaving only the larger ones exposed (fig. 144); evidently overgrowth is now proceeding more rapidly at the ventral than at the dorsal lip of the blastopore. At the close of the stage the greatly reduced yolk plug lies entirely on the postero-dorsal side of the lower pole of the vertical axis.

During the earlier part of the stage under consideration the closing fenestra often persists as a pit or small tract of distinct

Figs. 140 to 145 Dorsal views of embryos of *Cryptobranchus allegheniensis* in Stage 12, showing a series of stages in the development of the neural groove. Camera drawings from preserved material. The embryos are not oriented with respect to the vertical axis determined by gravity. $\times 7$.

Fig. 140 Showing earliest appearance of the neural groove.

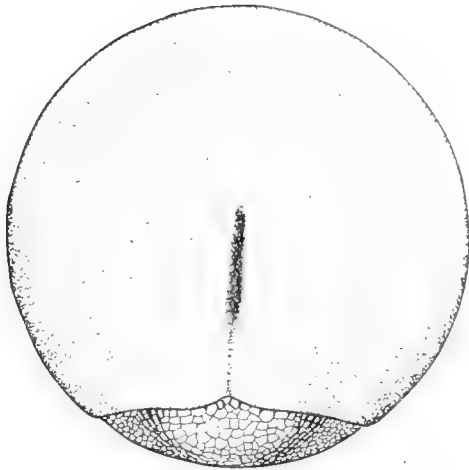
Fig. 141 Slightly later than the preceding.

Fig. 142 Slightly later than the preceding, showing segmented neural groove.

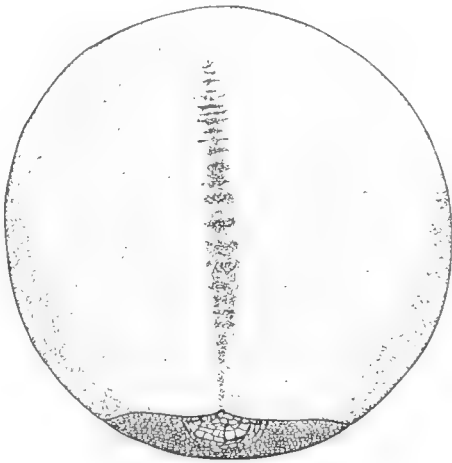
Fig. 143 Slightly later, segmented neural groove. See also figure 225 from the same embryo.

Fig. 144 Late neural groove.

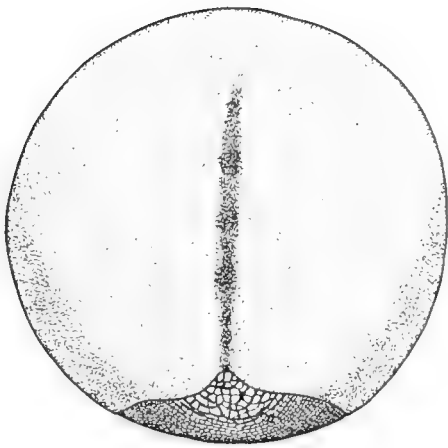
Fig. 145 Showing the condition of the neural groove at the time of the first faint indications of the neural folds.



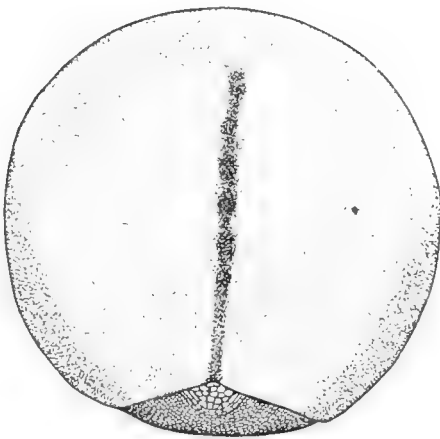
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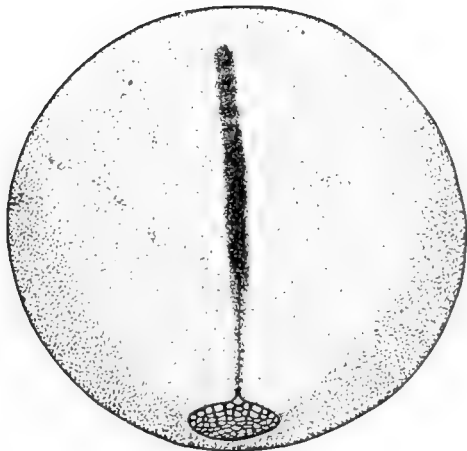
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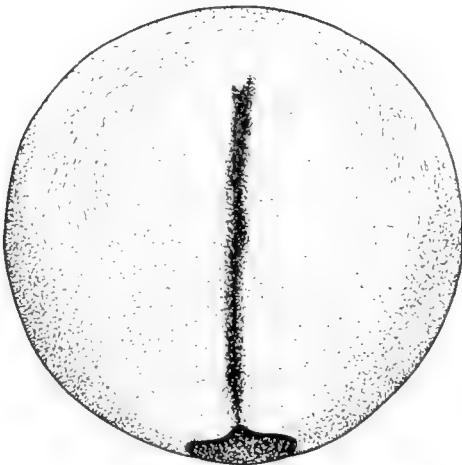
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furrows at the equator on the antero-ventral side of the egg. It usually disappears by the time the neural groove is well established.

In preserved material, the roof of the gastrocoele is considerably paler than the remaining surface of the egg; during the latter part of this stage the neural plate is usually differentiated as a spatulate area extending from the dorsal lip of the blastopore to a little distance in front of the upper pole of the vertical axis, and distinguishable through the greater whiteness of its surface (see especially figs. 223 and 224).

The dorsal lip of the blastopore is not a perfect arc of a circle, but is somewhat incurved on each side of a forward-extending notch in the median line (figs. 138 to 145).

At the time of its first appearance, the neural groove occurs as a distinct furrow extending *from the notch in the dorsal lip of the blastopore* forward in the median line for a distance of about 60 degrees; the anterior half is much broader and deeper than the posterior half (fig. 140). In a slightly later stage, the neural groove has extended to a total length of about 95 degrees but is nowhere so deep as in the anterior half during the preceding stage (fig. 141). It is now a rather shallow groove, narrow in its posterior portion, wider and more broken by occasional deeper depressions or fissures in its middle and anterior parts. These early transverse furrows do not occur at very regular intervals, and are probably only incidental to the process of infolding of the tissues.

A little later, the neural groove becomes decidedly deeper in its middle portion (fig. 142). The change is not uniform throughout this region, but instead there is a series of three or four large pits or depressions at fairly regular intervals, giving a segmented appearance to the groove. Sometimes this segmented condition is very marked; it has been repeatedly observed in living material. Gradually the segmented region, though less sharply marked becomes more extensive than before (fig. 143); it is best seen in living material viewed by transmitted light, when the neural groove appears made up of a regular succession of alternate light and dark areas. Shortly before the appearance of the neural

folds, the neural groove becomes conspicuous in its anterior as well as its middle portion by the broadening and deepening of the former region; at the same time the posterior end becomes fainter (fig. 144).

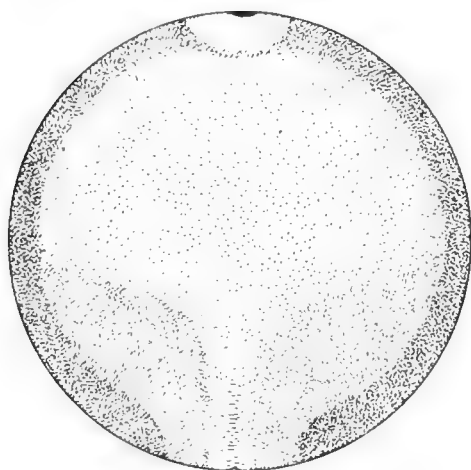
At the time of the first faint indications of the neural folds, the neural groove is both broad and deep throughout its entire length but especially in its anterior portion (fig. 145). During these later changes in the breadth and depth of the neural groove there has been very little increase in length; at the close of the period considered it has a length of about 105 degrees and extends from the dorsal lip of the blastopore nearly to the upper pole of the vertical axis.

According to Griggs ('10), in *Amblystoma* the first groove to appear in the median line of the neural plate is not the neural groove, properly speaking; this appears later on the same site. There first appears a 'posterior germinal depression' which does not reach the blastopore; a little later an 'anterior germinal depression' is formed, which is discontinuous with the earlier groove. In a later stage both give place to the neural groove which is a different structure on the same site.

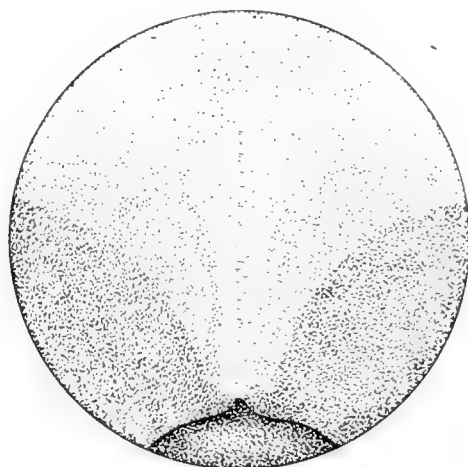
In *Cryptobranchus* the earliest groove to appear in the median line of the neural plate extends forward without break from the dorsal lip of the blastopore. The marked depression shown in figure 140 probably corresponds to the 'posterior germinal depression' in *Amblystoma*; the later depression in the anterior portion of the neural groove perhaps corresponds to the 'anterior germinal depression,' but it is at no time sharply separated from the remainder of the groove. The later history of the groove will be given in the following stages and should be consulted in this connection; but it may here be stated that after a careful study of both surface views and serial sections I have come to the conclusion that the differences in the grooves appearing early and late in the median line of the neural plate of *Cryptobranchus* are differences in degree, not in kind, hence I have used the term 'neural groove' throughout.

In living material the embryo may be viewed by transmitted light. During the early part of this stage (figs. 146 and 147)

the broad lateral bands lying in the posterior part of the egg at some distance from the median line are more marked than in the preceding stage. The study of sections shows that they are due to the combined optical effect of the mesoderm and an unusually thick region of the entoderm. The neural groove is particularly translucent. The greatly reduced blastocoele persists in the region of the equator on the antero-ventral side of the egg; the center of its external wall is marked by a pit, the vestige of the fenestra. During the latter part of Stage 12 (fig. 148) the



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Figs. 146 and 147 A living egg of *Cryptobranchus allegheniensis* in an early neural groove stage, viewed so far as possible by transmitted light. Figure 146 shows the upper hemisphere, figure 147 a postero-dorsal view. $\times 7$.

lateral bands are obscured by the thickening of the neural plate; in the central portion of the neural groove there usually appear a series of translucent pits arranged at regular intervals. The pit marking the site of the closing fenestra has disappeared, but there usually remains a translucent area indicating a vestige of the blastocoele; this area is often imperfectly separated from the translucent roof of the gastrocoele.

In this stage it is usually impossible to identify cleavage furrows in the yolk plug, but the stability of the larger cells and the fact that they remain longest exposed afford a means of locating approximately the vegetal pole. We have seen that in the preceding stage the vegetal pole was situated a little above the center of the area of largest macromeres; at the close of Stage 12 these

largest macromeres are the only ones exposed, and we may feel quite sure that the vegetal pole lies in their midst and probably very near the center of the greatly diminished yolk plug. The morphological axis of the egg is thus approximately determined by a line passing from the center of the yolk plug through the center of the egg, and at the close of Stage 12 this axis makes an angle of 52 degrees with the vertical—showing that rotation has proceeded 8 degrees further than in the preceding stage (fig. 149).

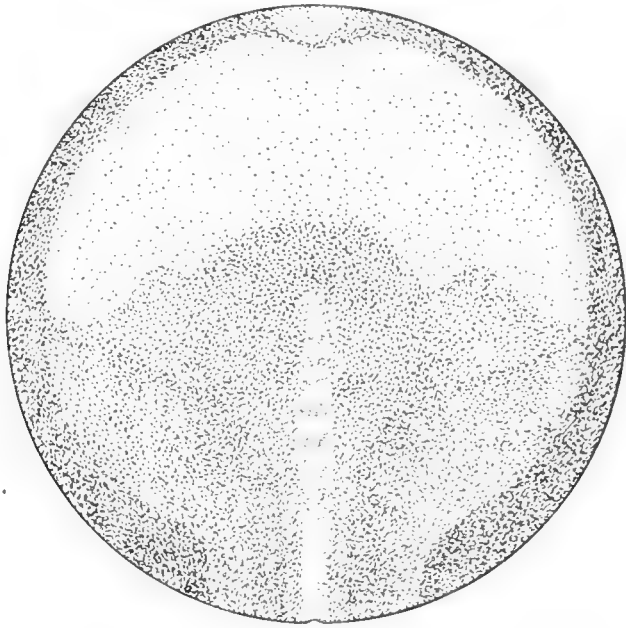
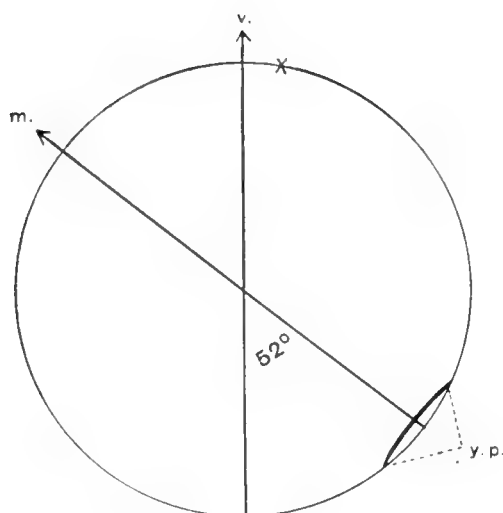
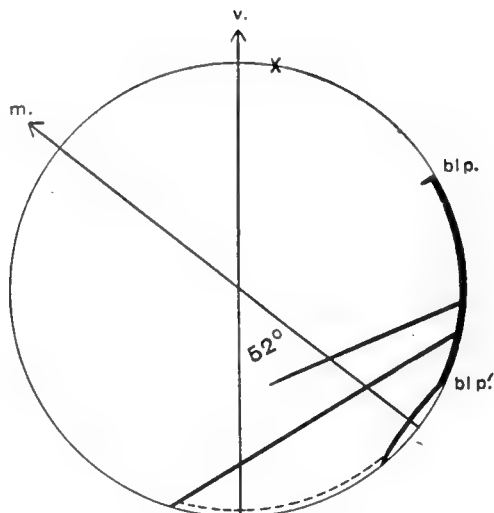


Fig. 148 Upper hemisphere of a living egg of *Cryptobranchus allegheniensis* viewed so far as possible by transmitted light, shortly before the appearance of the neural folds. $\times 9$.

It will be recalled that the dorsal lip of the blastopore first appears, on the average, 68 degrees above the vegetal pole (fig. 134), and that the ventral lip is first formed about 68 degrees from the vegetal pole on the opposite side (fig. 136). Hence the dorsal and ventral lips are formed respectively at approximately equal distances from the vegetal pole, though the ventral lip is formed much later than the dorsal. We may now compute the amounts of overgrowth of the dorsal and the ventral lips respectively: at the close of Stage 12 the dorsal lip has overgrown the yolk for an average distance of 55 degrees, and the ventral lip has advanced toward it through an arc of about 55 degrees;



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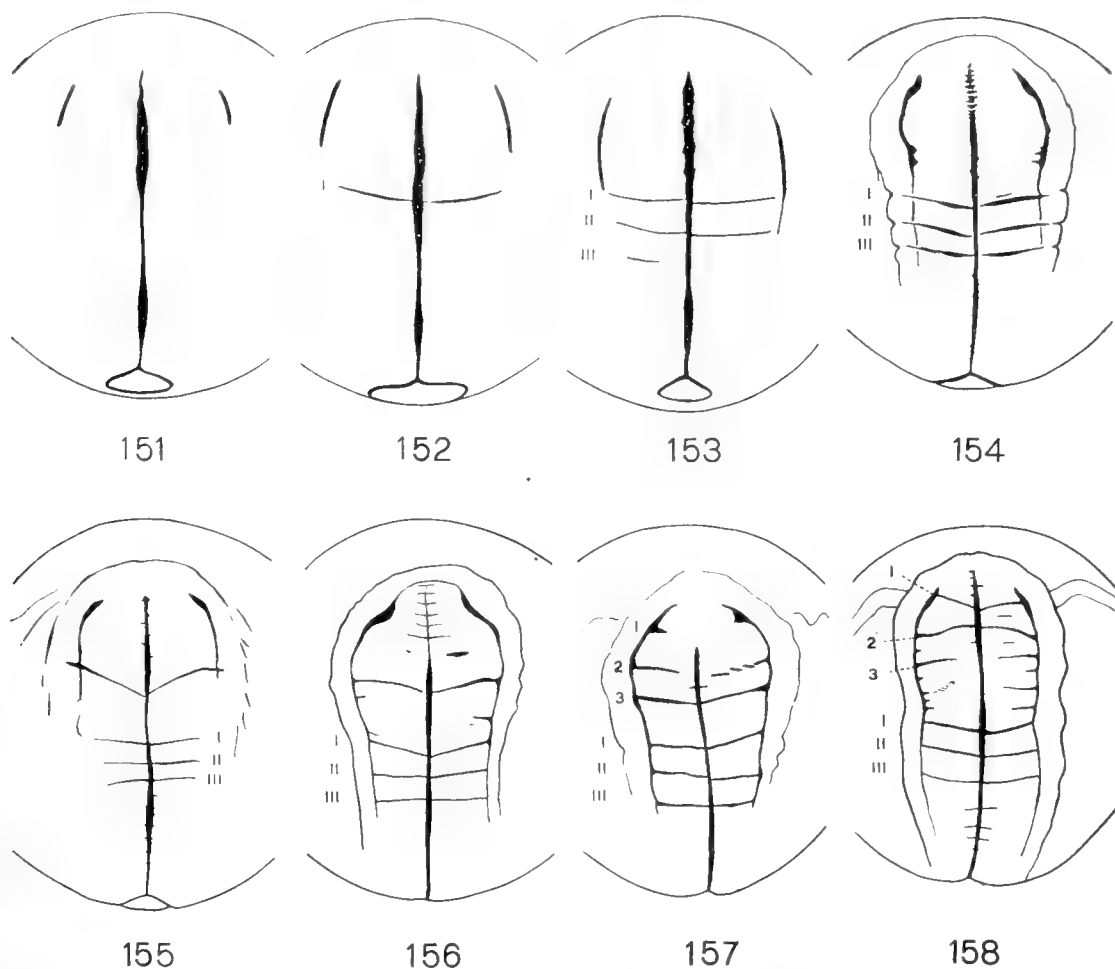
Fig. 149 Diagram of an egg of *Cryptobranchus allegheniensis* at the close of Stage 12, showing the amount of rotation and the position of the yolk plug; *m.*, morphological axis; *v.*, vertical axis determined by gravity; *y.p.*, yolk plug. The cross indicates the position of the anterior end of the neural groove.

Fig. 150 Combination of figure 144 with some features of figure 137, showing successive positions of the blastopore; *bl p.* and *bl p.'* respectively indicate early and late positions of the dorsal lip of the blastopore. The black band at the right of the figure indicates the amount of overgrowth (55 degrees) of the dorsal lip of the blastopore; the dotted line indicates the amount of overgrowth (55 degrees) of the ventral lip.

that is to say, the distances are approximately equal (fig. 150). By far the greater amount of overgrowth of the dorsal lip occurred during the preceding stage, hence it is clear that during the present stage overgrowth is taking place much more rapidly at the ventral than at the dorsal lip of the blastopore.

Stage 13: (figs. 151 to 164 and 226 to 228). The most conspicuous changes during this stage are those concerned with the formation of the neural folds and the segmentation of the neural plate. The neural folds begin to form about one and one-half days after the appearance of the neural groove. During the progress of this stage the neural groove becomes most conspicuous in an anterior and a posterior portion, separated by a middle region in which it is comparatively faint (see especially figs. 151 to 155).

The early stages in the formation of the neural folds are shown in figures 151 to 158 and need no further description, save to men-



Figs. 151 to 158 Stage 13. A series of embryos of *Cryptobranchus allegheniensis* showing early stages in the formation of the neural folds and the segmentation of the neural plate. Camera drawings, finished under the binocular, from preserved material. I, II and III indicate the earliest transverse grooves; 1, 2, 3, grooves appearing a little later, numbered consecutively and not in the order of appearance. $\times 6$.

tion that the surface just outside of the neural folds becomes very much roughened and traversed by fissures parallel to the folds, indicating stresses and the rapid shifting of material. During the later part of this stage a pair of less conspicuous transverse folds appear lateral to the anterior end of the neural plate (fig. 158); the significance of these folds has not yet been determined with certainty (but see Stage 15).

The first transverse groove to cross the neural plate is shown in figure 152. A little later, two transverse grooves appear in rapid succession posterior to it. These first three transverse

grooves are equidistant, and so distinct that they may readily be seen with the naked eye; since they regularly appear in the same order and position in different embryos, and persist throughout the further history of the open neural plate, they serve as trustworthy landmarks during the following stages. In the figures they are numbered with Roman numerals. By following their history through later stages they have been traced to the region of the medulla oblongata of the adult brain; consequently, at least all that portion of the neural plate in front of Groove III belongs to the cephalic plate.

The early segmentation of the cephalic plate in front of Groove I will now be considered. There first appears a transverse groove dividing this region into two portions of which the posterior is slightly the smaller (figs. 155 and 156); the anterior of these areas is then crossed by two more grooves (figs. 157 and 158), while the posterior area is for the present doubtfully segmented. The smaller transverse grooves occurring in various parts of the cephalic plate are irregular in position and probably are of no segmental value; most of them disappear in later stages. Those grooves in front of Groove I which are regarded as of metameric value are numbered with Arabic numerals, consecutively and without regard to the order of appearance.

The question naturally arises whether these early transverse divisions of the cephalic plate are neural in origin or secondarily produced by the segmentation of the underlying mesoderm. This question has not yet been thoroughly investigated by the study of sections, but the results of a preliminary examination favor the idea that in front of Groove I at least, they are primarily neural structures; the mesoderm, particularly in front of Groove I, is at this time quite thin as compared with the neural plate, and hardly capable of producing the modifications of the latter layer.

Since Grooves I, II, III, etc. (see also Stage 14) are produced in regular order from before backward there is ground for suspicion that they are intimately connected with the formation of the mesoblastic somites. In view of the fact that the segmentation of the region immediately in front of Groove I is late in

appearing and seldom clearly expressed (Stage 14), we must be on our guard against a possible discontinuity or difference in kind between the segmentation of the anterior and the posterior regions of the cephalic plate. These points can be settled only by a careful study of sections of eggs that have first been described externally; but from surface views alone we are justified in claiming that we have in the open cephalic plate transverse divisions which may be homologized in different embryos, and which are probably of true metameric value; hence they may be of use in solving the vexed problem of the segmentation of the vertebrate head.

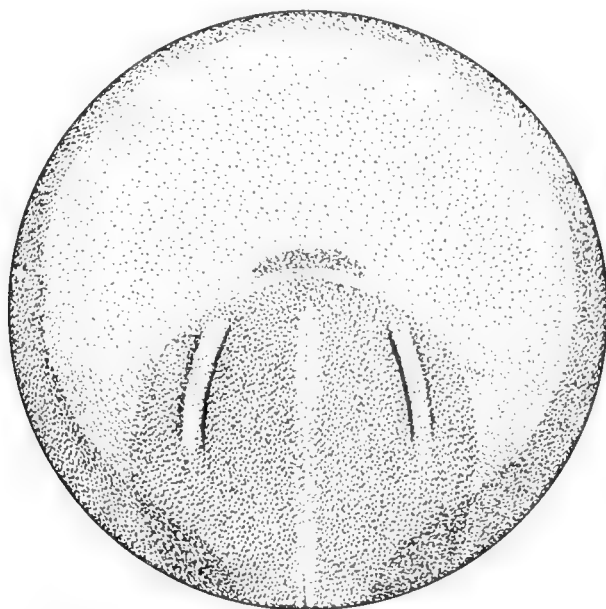


Fig. 159 A living embryo of *Cryptobranchus allegheniensis* in the early part of Stage 13, viewed in direct sunlight, and so far as possible by transmitted light. From a freehand sketch of the upper hemisphere. $\times 10$.

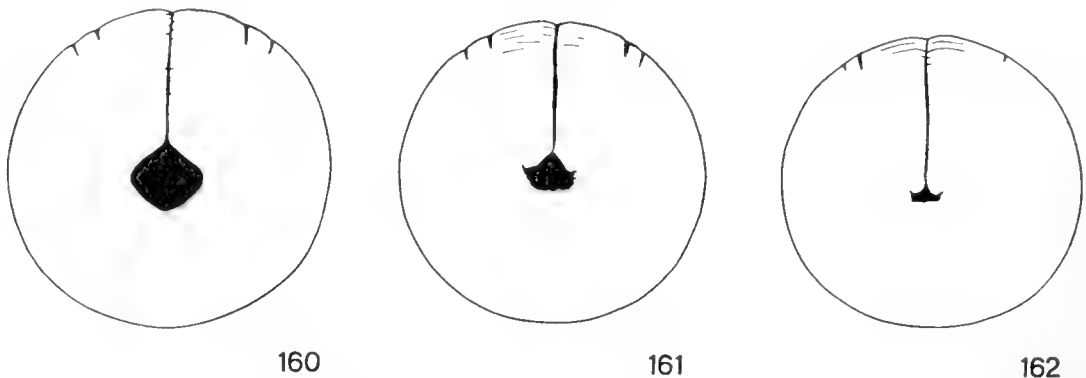
A pair of depressions just within the neural folds near the anterior end of the cephalic plate probably indicate the anlage of the optic vesicles (cf. Eycleshymer '95; Locy, '95).

Some features of this stage are best brought out by the study of living material; for this purpose embryos have been examined in direct sunlight. As shown in figure 159 a transverse opaque band early appears directly in front of the neural plate in the median region; in position and appearance it reminds one of the ectamnion of the chick (Lillie '08, pp. 138 and 139). The neural

folds are conspicuous at an earlier stage in living than in preserved material. In embryos later than the one figured, transverse furrows in the neural plate appear as described in preserved material.

During Stage 13 the blastopore nearly closes, then makes little advance in this respect during the next two stages. Variations in the degree of reduction of the blastopore during these three stages are so great that this structure cannot be used as a character for classifying embryos into stages.

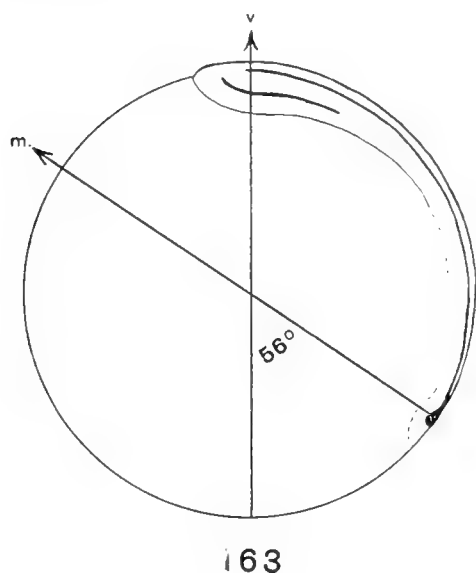
As shown in figures 160 to 162, during Stage 13 the blastopore changes from a diamond shape to that of an anchor; the forward-



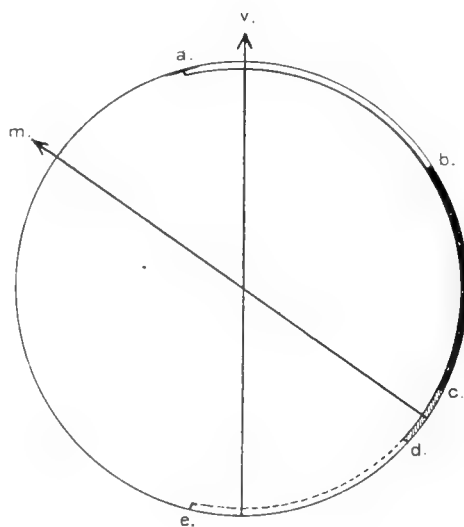
Figs. 160 to 162 A series of embryos of *Cryptobranchus allegheniensis* in Stage 13, showing changes in the size and form of the late blastopore. Camera drawings from preserved material. $\times 5$.

projecting part is derived through an exaggeration of the notch previously noted in the dorsal lip of the blastopore. The lappets lying on each side of this median notch of the blastopore are continuous with the neural folds; through their apposition the dorsal part of the yolk plug becomes closed over. Thus the extreme posterior end of the embryo is undoubtedly formed by a process of concrecence. As shown in later stages, the ventral part of the blastopore becomes reduced to a transverse slit (figs. 177 and 178); during this process the yolk plug usually becomes entirely withdrawn into the egg, but a small mass of yolk sometimes persists at the surface. The late history of the blastopore is much the same in *Cryptobranchus japonicus*, as described by Ishikawa ('08).

At the close of Stage 13 the neural groove has reached a length of about 124 degrees; its anterior end usually lies quite accurately at the upper vertical pole, while the neural folds extend about 16 degrees in front of it. We have seen that the closing blastopore marks the approximate position of the vegetal pole; this pole has now rotated a total distance of 56 degrees from the vertical axis.



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Fig. 163 Diagram of an embryo of *Cryptobranchus allegheniensis* at the close of Stage 13, showing the position of the neural plate and neural folds with reference to the morphological and the vertical axes; *m*, morphological axis; *v*, vertical axis.

Fig. 164 Diagram showing the position of the embryonic body of *Cryptobranchus allegheniensis*, and illustrating some features of embryo-formation; *a* to *b* (72 degrees), portion of the embryo formed in situ; *b* to *c* (60 degrees), portion formed by overgrowth of the dorsal lip of the blastopore, with the possibility of concrescence; *c* to *d* (roughly estimated at 16 degrees), portion undoubtedly formed by concrescence; *d* to *e* (60 degrees), distance traveled by the ventral lip of the blastopore. Other lettering as in the preceding figure.

We now have sufficient data for a statement of the position of the embryonic body on the egg, and for pointing out certain features of its mode of formation (figs. 163 and 164). About 72 degrees of the anterior end of the embryo is formed in situ. About 60 degrees is formed in connection with the overgrowth of the dorsal lip of the blastopore; in this case there is the possibility of concrescence through the apposition of material on each

side of the median notch, which may be shifted toward the median line during the process of overgrowth. This point can be definitely settled only by experiment; but in the absence of experimental data we can say that there is no positive evidence of such a process taking place, while certain considerations weigh against it. For in certain observed cases rapid shifting of material is accompanied by a roughening of the surface with the formation of parallel fissures, as in the region just outside of the neural folds during their formation and progress toward the median line. There is an entire absence of any such feature in the dorsal lip of the blastopore.

A region at the posterior end of the embryo, which is roughly estimated at 16 degrees, is formed through the concrescence of the lateral and ventral lips of the blastopore. A part of this material has been brought through a distance of 60 degrees by the overgrowth of the ventral lip of the blastopore; it will be observed that this distance equals that of the overgrowth of the dorsal lip of the blastopore.

At the close of Stage 13, when the embryonic body is for the first time clearly indicated, it has a total length of about 148 degrees. The posterior end is formed around the vegetal pole; the anterior end lies about 40 degrees from the animal pole. Hence the statement made in Part I (Smith '12) to the effect that the axis of polarity of the late ovarian egg defines the principal axis of the embryo is not quite accurate; but the embryo is formed almost wholly in a hemisphere of the egg lying to one side of the axis of polarity. A review of its history shows that the embryo is formed almost entirely out of material derived from a band of cells lying in the equatorial region of the late blastula and early gastrula, and that this band of cells is narrow on the ventral, broad on the dorsal side of the egg (figs. 116 and 123).

Goodale ('11), after reviewing the literature of the subject in connection with his own work on *Spelerpes*, concluded that

The amphibian embryo develops almost entirely in a vertical half of the egg, the tail appearing near the lower pole, while the anterior end of the body develops in greater or less degree in the upper hemisphere,

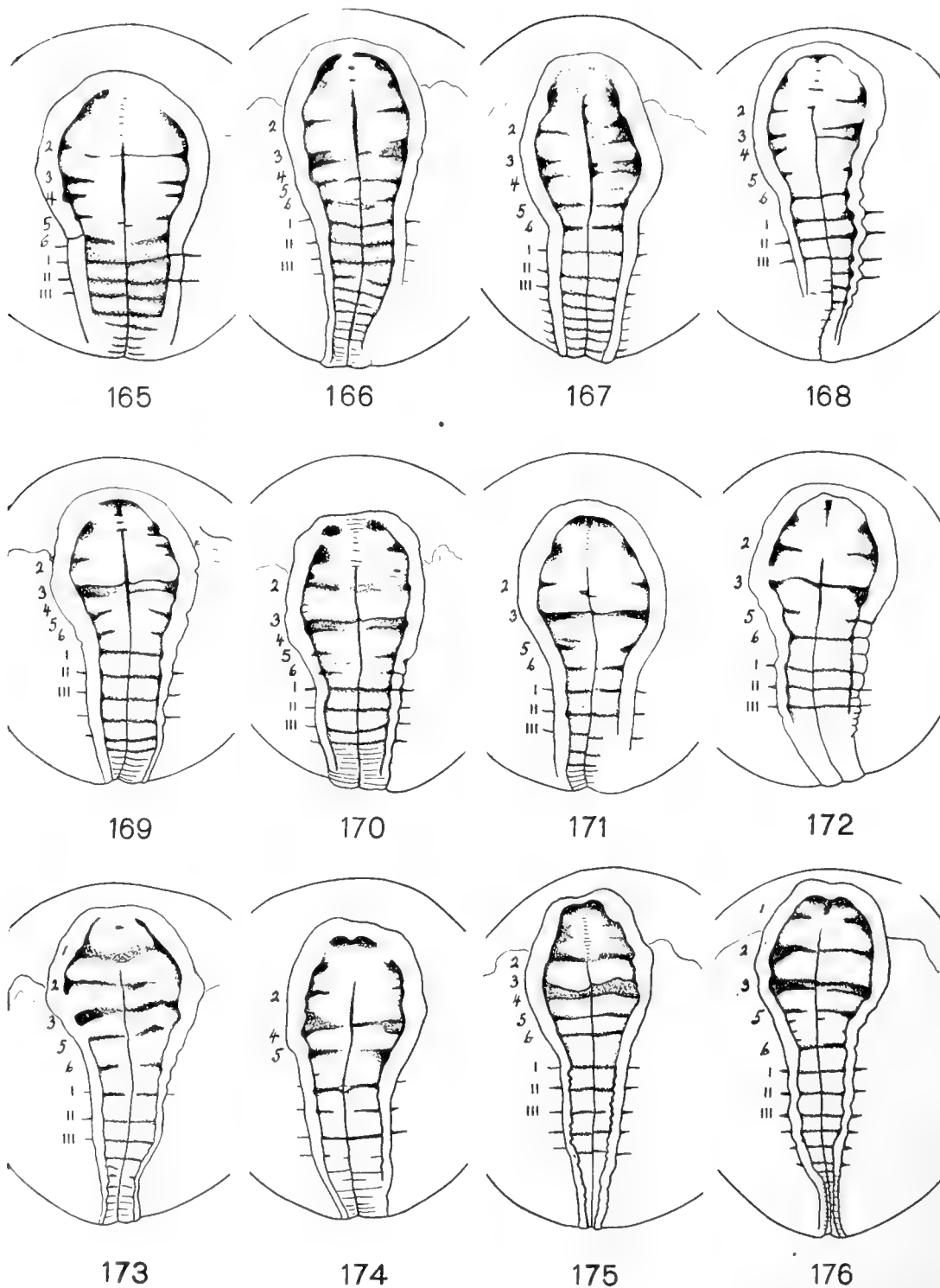
depending upon the particular species. The position of the head of the embryo seems correlated with the length of the embryo, so that the longer the embryo, the higher up on the egg it develops.

The terms 'upper' and 'lower' are evidently here used in the sense of animal and vegetal, that is to say, with reference to those points on the surface of the egg which were in the vertical axis of the egg before it commenced to rotate; therefore the results obtained with *Cryptobranchus* fall in line with the general statement quoted. The results of Goodale on *Spelerpes* and my own work on *Cryptobranchus* agree closely in locating the posterior end of the embryo at the vegetal pole; it is worthy of note that this conclusion was reached independently and by entirely different methods.

Stage 14: (figs. 165 to 179 and 229 to 232). This stage is reached about one day later than the beginning of Stage 13. Since at this time scarcely any two embryos agree in the rate of development of homologous regions of the body, it is impossible in this stage to make a close classification. In marking off this stage from the one following, the principal character considered is the approach of the neural folds toward the median line.

Figures 165 to 176 represent twelve embryos that illustrate the principal changes in the antero-dorsal region during this stage. It will be seen that there is a progressive addition of transverse grooves posterior to the three that first appeared. In front of Groove I the cephalic plate is traversed primarily by six grooves; of these Groove 1, which was noted in the preceding stage, has a very transitory existence and in most cases is lost in Stage 14; likewise the median portion of Groove 2 has often disappeared. Moreover in this or the following stage Groove 4 disappears, following a marked depression and perhaps submergence of the segment between it and Groove 3.

A significant relation exists between Grooves I, II, III, etc., and the intersomitic grooves which now appear just outside the neural folds; by an inspection of figures 165 to 176 it will be seen that in all cases these are in direct apposition. Since the mesoblastic somites are the most characteristically segmented structures of the vertebrate body, it follows that the true segmental units of



Figs. 165 to 176 Antero-dorsal views of embryos of *Cryptobranchus alleghe-ni-ensis* in Stage 14, showing especially the segmentation of the neural plate. Camera drawings finished under the binocular, from preserved material. $\times 6$.

The earliest transverse grooves to cross the neural plate are numbered with Roman numerals in the order of appearance; in front of Groove I the transverse grooves are numbered with Arabic numerals consecutively without regard to the order of appearance. Figure 166 is from the embryo photographed for figures 229 and 230; figure 176 is from the embryo photographed for figures 231 and 232.

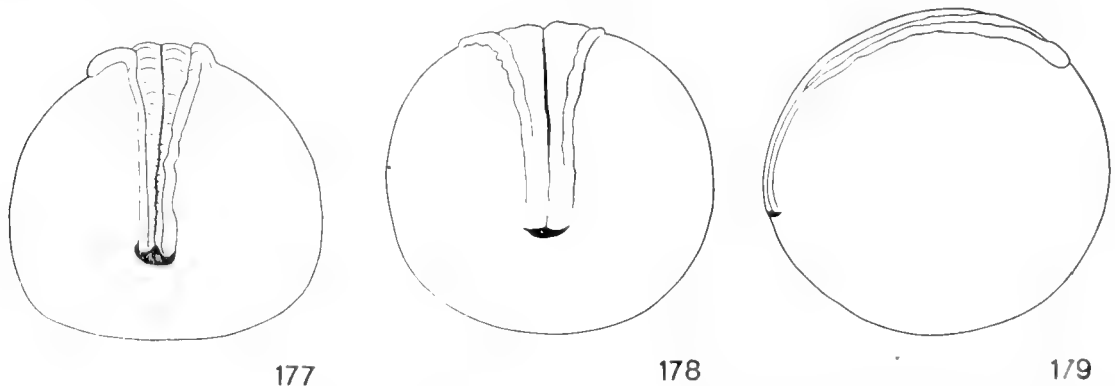
the open neural plate, in this region at least, are the divisions between grooves—that is, the ridges rather than the depressions, for the former are in line with the body somites. If, as appears likely, there is continuity between the structures of the anterior and posterior regions of the cephalic plate, then the rule may be extended to include the entire neural plate. As thus defined, there are seven segments—‘neuromeres’—in front of Groove I; posterior to this groove an undetermined number of segments also belong to the head.

In the early stages of the formation of the neural folds transverse grooves are sometimes found in them, continuous with the transverse grooves of the neural plate (see especially figs. 154, 165 and 172). In such cases the neural fold is marked by an outer as well as an inner notch, both in line with the transverse furrow of the neural plate. This condition is only temporary and apparently it is transitional to a later phase in which the inner notch grows at the expense of the outer one, until an outer convexity of the fold appears opposite the inner concavity (see especially fig. 168). In the region of the body somites these outward flexures thus lie in line with the intersomitic grooves as well as with the transverse grooves of the neural plate. This condition is seldom so well expressed as in the embryo shown in figure 168; the convolutions of the neural folds are often irregular and bear no definite relation to the segments. But it is fairly certain that in all cases where the neural folds are well upraised and flexures occur which are segmentally arranged, the outward flexures lie opposite the transverse furrows and not opposite the ridges between them. Moreover, in sagittal sections the transverse grooves on the external surface of the neural plate are found to correspond to ridges on the internal surface.

Those who have described segmental structures in the neural folds or closed neural tube have, as a rule, accepted Orr's ('87) definition of the segmental units or neuromeres as *outward* flexures of the neural folds. But if the above considerations be well founded, the true segments are to be sought rather in the segments between the transverse grooves of the neural plate, and in the *inward* flexures of the neural folds. In other words neu-

romeres are the transverse ridges on the inside rather than on the outside of the brain. To a limited extent this view coincides with that of Kupffer ('85 to '93), who maintained that the true neuromeres are the transverse divisions of the open neural plate rather than the later appearing structures in the neural folds.

Most of the features of this stage thus far described have been observed in living as well as in preserved material. The literature on the early development of the central nervous system has recently been reviewed by Griggs ('10); a more comprehensive



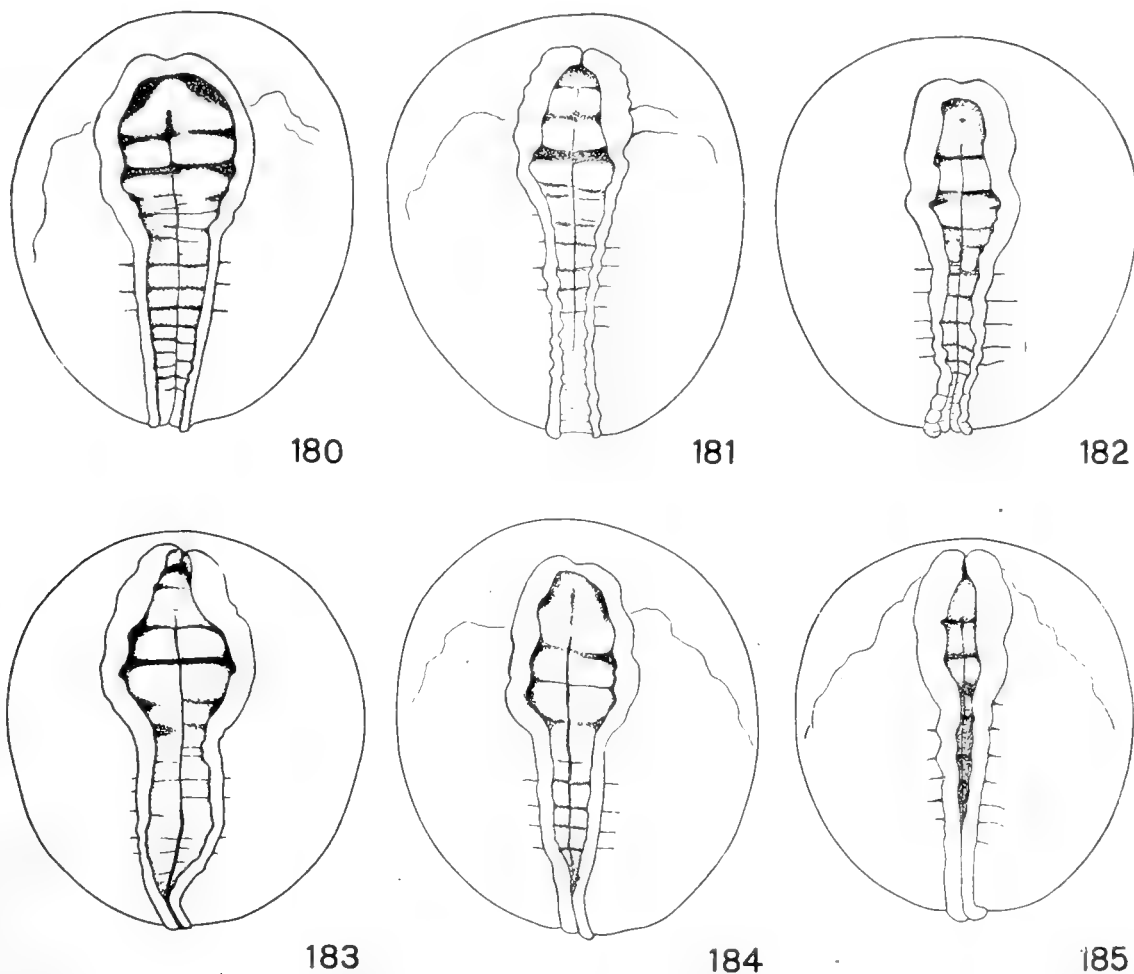
Figs. 177 to 179 Camera outlines of embryos of *Cryptobranchus allegheniensis* in Stage 14, drawn from preserved material. $\times 5$.

Figs. 177 and 178 Posterior views showing late blastopore.

Fig. 179 Lateral view showing position of the embryonic body at the close of Stage 14. The egg is shown in its natural position with respect to the vertical axis, which passes in the plane of the paper parallel to its lateral margins. The embryo proper has a total length of about 155 degrees. This figure and figure 166 are drawn from the same egg.

survey of the earlier work on the segmentation of the vertebrate head is given by Locy ('95). Ishikawa ('08) has described segmental divisions in the open neural plate of *Cryptobranchus japonicus*.

During this stage, if not already in the preceding stage, the anterior or dorsal part of the blastopore becomes closed over, while the ventral part persists as a transverse crescentic slit (figs. 177 and 178). At the close of Stage 14 the embryo has increased slightly in length (fig. 179); it now extends over about 155 degrees of the surface of the egg. This increase in length



Figs. 180 to 185 Antero-dorsal views of embryos of *Cryptobranchus alleghe-niensis* in Stage 15. Camera drawings finished under binocular, from preserved material. Figure 182 is drawn from the embryo photographed for figure 234; figure 184 is drawn from the embryo photographed for figure 233. $\times 6$.

of the embryo involves a noticeable increase in the antero-posterior dimension of some of the neuromeres.

Stage 15: (figs. 180 to 189; 233 and 234). This stage is reached about eighteen hours later than the beginning of the preceding stage.

In the following account, each neuromere is designated by the number of the groove bounding it on the posterior side. Neuromeres 1 and 2 have usually coalesced; neuromere 4 disappears during this, if not in the preceding stage. More definite swellings now occur in neuromeres 1, 2, 3 and 5; the region between Grooves 5 and I is less clearly segmented and is usually somewhat depressed. The outlines of the neural folds in the head region

now suggest the definitive primary divisions (forebrain, midbrain and hindbrain) of the embryonic brain.

The various structures of the neural plate have not yet been followed into the definitive divisions of the embryonic and adult brain; but the preliminary examination of some later embryos dissected by splitting them in the median line with a razor shows that the transverse divisions in the neural plate persist for some time after the closure of the neural folds. Neuromeres in the closed neural tube are also often apparent from the surface. Hence it is easy to judge approximately concerning the fate of individual neuromeres of the cephalic plate, but to avoid possible error it seems best to defer a definite statement until the internal history of the brain has been more carefully studied.

The pair of folds which in the preceding stages extended transversely on each side of the cephalic plate now slant backward (see especially fig. 185); the appearance, particularly in living material, suggests that they are in some way concerned with the origin of the vascular bands which in later stages extend along each side of the yolk sac and give rise to the omphalomesenteric or vitelline veins (fig. 192).

The transverse opacity in front of the neural plate is conspicuous in living material viewed by transmitted light (fig. 186), but is not apparent in surface views of preserved material.

The anterior part of the blastopore is now normally closed over, and the posterior or ventral part is reduced to a transverse slit (figs. 188 and 189). Apparently the middle portion of this transverse slit never becomes completely closed, but in later stages persists as the anal or cloacal opening. The embryonic body has elongated so that it now extends over about half the circumference of the egg (fig. 187).

For the study of transverse divisions in the open neural plate, *Necturus* is not nearly so favorable as *Cryptobranchus*. In *Necturus* the blastopore (figs. 268 to 279) closes much earlier than in *Cryptobranchus*. Moreover in *Necturus* the closure of the blastopore is often practically complete; in many specimens preserved at the time of the closure of the neural folds, scarcely more than a vestige of the blastopore is visible from the surface.

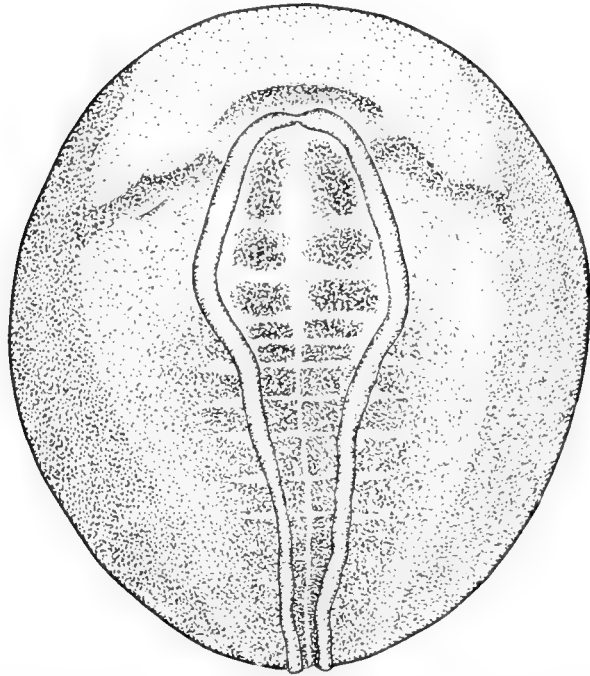
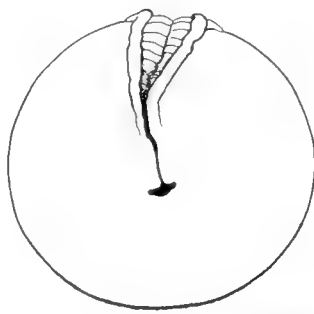


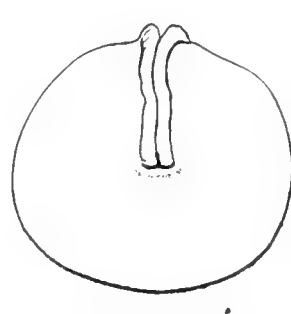
Fig. 186 Antero-dorsal view of a living embryo of *Cryptobranchus allegheniensis* in Stage 15, viewed mainly by transmitted light. From a freehand sketch. $\times 10$.



187



188



189

Fig. 187 Lateral view of an embryo of *Cryptobranchus allegheniensis* in Stage 15, showing the position of the embryonic body. The egg is shown in its natural position with respect to the vertical axis which passes in the plane of the paper parallel to its lateral margins. Camera drawing from preserved material. $\times 5$.

Figs. 188 and 189 Posterior views of embryos of *Cryptobranchus allegheniensis* in Stage 10, showing the form of the late blastopore. Camera drawings from preserved material. $\times 5$.

Figs. 183, 187 and 188 All drawn from the same embryo.

Figs. 185 and 189 Drawn from the same embryo.

In these later stages, the blastopore is doubtless often indistinguishable in living material (figs. 274 to 276).

A triradiate form of the blastopore is not so frequently found in *Necturus*; in *Cryptobranchus japonicus* (Ishikawa '08), it often occurs. A general resemblance may be noted between the blastopore of the urodeles cited and that of the dipnoans (*Ceratodus*, Semon '01; *Protopterus* and *Lepidosiren*, Kerr '09).

B. Summary

Gastrulation involves a combination of the processes of invagination or emboly, and overgrowth or epiboly.

During gastrulation the roof of the segmentation cavity becomes very thin, and is bounded superficially by a sharp furrow, the 'septal furrow' of Ishikawa.

On account of the translucent character of certain parts of the egg, many of the internal changes concerned with gastrulation can be followed quite satisfactorily in living material.

For some time after the beginning of gastrulation, the vegetal pole may be located through the intersection of the first and second cleavage furrows.

During gastrulation and the formation of the neural groove and neural folds the egg rotates on an axis at right angles to the median plane so as to bring the morphological axis at an angle of 56 degrees from the vertical.

The dorsal lip of the blastopore is formed about 68 degrees above the vegetal pole; the ventral lip is formed much later at an equal distance on the other side of the vegetal pole. Since the closing blastopore lies approximately at the vegetal pole, overgrowth proceeds through equal distances on the dorsal and the ventral sides of the egg. During the early part of gastrulation, before the ventral lip is formed, overgrowth takes place rapidly and extensively at the dorsal lip; after the blastopore has become a complete circle, overgrowth takes place very slowly at the dorsal lip, very rapidly at the ventral lip.

Not until after the neural folds are well formed is the yolk plug completely overgrown; as compared with *Necturus* and

most amphibian eggs the blastopore is very late in closing. In late stages the blastopore has the form of an anchor or an inverted *T*; the posterior transverse portion remains longest as an open slit, and the center of this transverse portion never completely closes but persists as the anal or cloacal aperture.

The posterior end of the embryo forms approximately at the vegetal pole. At the time when the neural folds are first formed the embryo has a total length of about 148 degrees, hence its anterior end does not reach the animal pole. About 72 degrees of the anterior end of the embryo (nearly half its total length) is formed in situ; about 60 degrees posterior to this is formed by overgrowth with the possibility of concrescence. Only a very small part at the posterior end, perhaps 16 degrees, is formed by the meeting of the lateral and ventral lips of the blastopore; this part is undoubtedly formed by concrescence.

From the time of its first appearance the neural groove is continuous with a median notch in the dorsal lip of the blastopore. There is evidence that the neural groove early acquires a segmented structure.

Transverse grooves, definite in number and location, cross the neural plate, dividing it into true segments or neuromeres. In the region of the mesoblastic somites the transverse grooves of the neural plate are in line with the intersomitic grooves, and the neuromeres are in line with the somites. Segmental flexures of the neural folds sometimes occur; in these cases the outward flexures of the neural folds are in line with the transverse grooves, and the inward flexures are in line with the neuromeres.

At the time of the closure of the neural folds, the embryo has increased in length so that it extends over about one-half of the circumference of the egg.

IX. DEVELOPMENT AFTER THE CLOSURE OF THE NEURAL FOLDS

A. Description by stages, to the time of hatching

Most of the important features of the later external development are sufficiently illustrated by the photographs. Only a brief account is here necessary and this will deal principally with

observations on living material. Some comparisons with *Necturus* have been given in a previous paper (Smith '11 a). Late stages of *Cryptobranchus japonicus* have been figured by Ishikawa ('04 and '08) and de Lange ('07).

Stage 16: (figs. 235 to 237). This stage is reached about eighteen hours later than the beginning of Stage 15. It is characterized by closed neural folds which are still more or less separated by a median groove. Ganglionic ridges are forming at the sides of the brain. The blastopore is no longer a transverse slit, but a small round orifice which probably represents the definitive cloacal opening. Up to this time the great majority of the eggs have retained the 'vitelline membrane.' During Stage 16 or slightly later this covering usually becomes ruptured as a consequence of the growth of the embryo and is finally cast off.

During the gastrula and open neural groove stages, careful observations have been made to test the presence of cilia on the ectoderm, with absolutely negative results. Currents of water produced by ciliary motion may be detected through the movements of yolk particles within the vitelline membrane when this is present, or by means of powdered carmine added to the water in cases where the vitelline membrane has been shed. At the time when the neural folds are closing, cilia are present on the sides of the body and the ventral surface of the yolk sac, but are absent from the neural folds. The general direction of the ciliary currents is toward the posterior end of the body.

Stage 17: (figs. 238 to 242). This stage is reached about a day later than Stage 16. The neural folds are definitely closed and the head well upraised. The optic vesicles are indicated by slight paired expansions of the anterior part of the brain. In some embryos the anlage of the pronephros is apparent through an elevation of the overlying ectoderm. Cilia are absent from the dorsal surface above the neural tube but are quite generally present elsewhere and are particularly strong or numerous on the dorsal surface of the body, lateral to the neural tube. In general the beat of the cilia along the sides and ventral surface of the body is backward.

The embryo is still erect (i.e., with the dorsal surface uppermost). In this position it has been observed, in many cases, to rotate slowly on a vertical axis. To test the direction of rotation a large number of embryos were placed separately in watch glasses and individual records made. Out of sixteen embryos that showed rotation, only two moved in a clockwise direction, the other fourteen in an anti-clockwise direction. The rotation is, of course, caused by the cilia. The direction of rotation in this stage can hardly be explained as the result of a tendency for the embryos to lean to one side oftener than to the other, for, as will be shown in a later stage, the facts are otherwise. Possibly more extended observations would show more equality in the results; or there may be a uniform asymmetry in the distribution, or in the rate or direction of beating, of the cilia of the ventral surface of the yolk sac.

Stage 18: (figs. 243 and 244). This stage is reached about twenty-four hours after the beginning of Stage 17. It is characterized by a prominent outstanding head with marked cephalic flexure and distinct optic vesicles, and by the presence of the pronephros and the first definite indications of the budding tail. During the latter part of this stage the mandibular arch is usually recognizable.

Patches of cilia are now distributed over the entire surface; the beat of the cilia is in general backward and the currents are much the same as figured in the next stage (fig. 190).

During this stage the embryo topples over from its erect position so as to fall to one side, on which it lies throughout several succeeding stages until spontaneous movements enable it to change its position. An incidental result of this position is to bring a larger area of the ciliated surface into contact with the substratum; as a consequence of this and of the stronger ciliation, rotation of the embryo is now of more marked occurrence. The most rapid motion observed was performed by an embryo that completed a rotation in just two minutes.

The functional value of the ciliary motion is at least two-fold: (1) it bathes the surface of the embryo with currents of water which are subservient to respiration; and (2) rotation of the

embryo, when it occurs, serves to prevent adhesion of the embryo to the envelope with consequent abnormalities.

The ciliation and rotation of the frog embryo have been described by various writers, notably Assheton ('96). Piersol ('09) has described rotation in the embryo of *Plethodon*.

Stage 19: (figs. 190 and 245 to 248). This stage begins about twenty-four hours later than Stage 18. It is characterized by from two to three distinct gill invaginations, a budding tail, a very marked outward expression of the pronephros (see especially figs. 245 and 246), and beginning lateral vascular bands, the anlage of the vitelline veins (see especially fig. 245). In addition

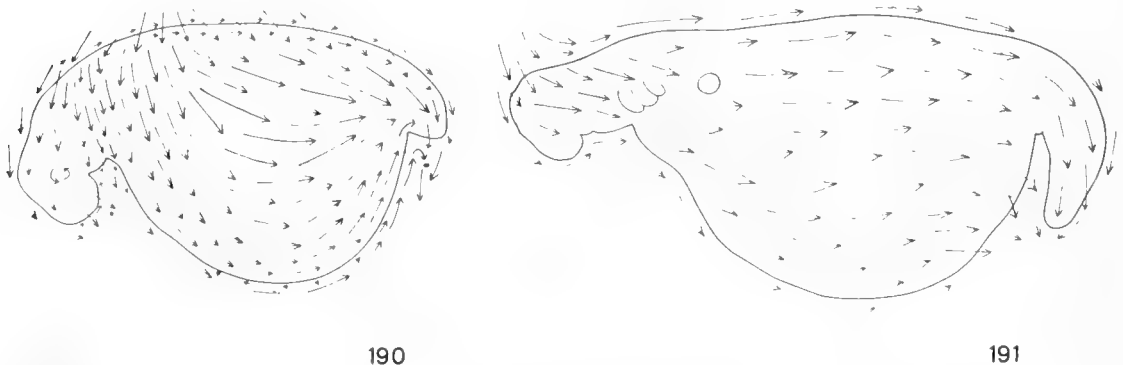


Fig. 190 Diagram of an embryo of *Cryptobranchus allegheniensis* in Stage 19, showing the direction of the water currents produced by cilia.

Fig. 191 Same as figure 190, for Stage 21.

to the cephalic flexure there is a slight cervical flexure which reaches its maximum in this stage. About sixteen to twenty mesoblastic somites are apparent in surface views.

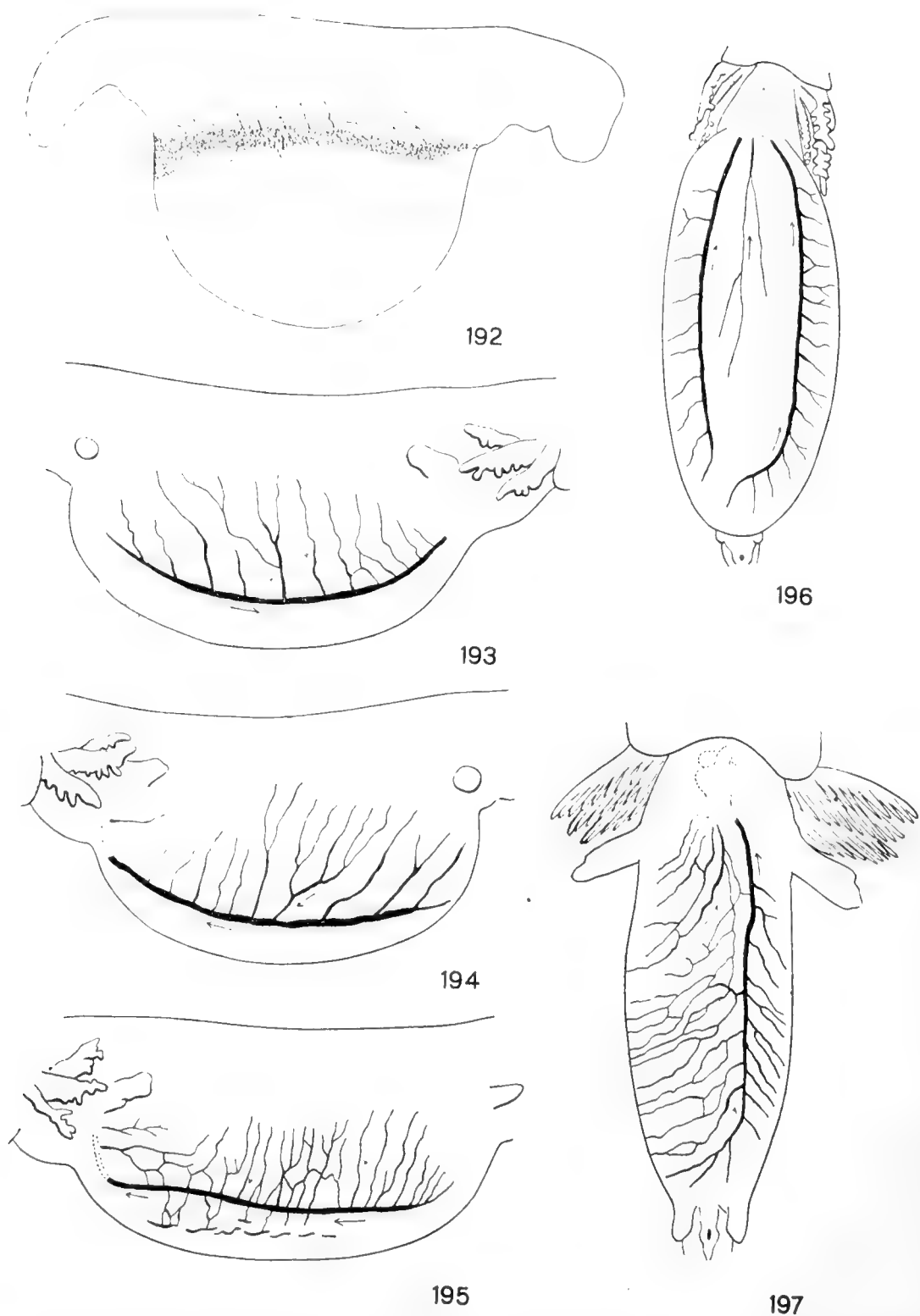
In the living embryo, the lateral vascular bands are conspicuous structures, for they are pink with blood; but they are not yet differentiated into true veins. Overlying the upper part of the yolk sac, they extend from the heart region longitudinally on each side of the body and meet posteriorly a little below the tail. During this stage and the stages immediately following, they shift slowly toward the ventral surface. There is considerable variation in the position of this band in embryos that are otherwise in the same stage. A similar vascular area has been figured for *Cryptobranchus japonicus* by de Lange ('07).

In this stage practically all embryos are found lying on either the right or the left side. To determine the relative number of cases of each, one hundred and nine embryos, taken at random from several different spawnings, were examined; fifty were found to lie upon the right side, fifty-nine upon the left side. These numbers are approximately equal, hence the occurrence follows the laws of chance.

Rotation of the embryo now occurs in a greater proportion of cases than in the preceding stage, though of the embryos studied hardly more than one in ten has been observed to rotate. It is noticeable that in some cases the direction of rotation is clockwise, in other cases anti-clockwise. In eleven embryos that showed rotation eight moved in an anti-clockwise direction; all these were lying on the left side. Three embryos rotated in a clockwise direction; of these two were lying on the right side, one on the left side. Hence as a general rule the direction of rotation is correlated with the position of the body. The actual direction is not what one would expect if the beat of the cilia were directly backward in all parts of the body; but by an inspection of figure 190 it will be seen that in the head region the beat is ventrad, in the posterior region dorsad. The ciliary currents are alike on the two sides of the body.

Stage 20: (figs. 192 and 249 to 251). This stage is reached about two days later than the beginning of Stage 19. Through the loss of the cervical flexure the head is now brought more nearly in line with the body, but on the other hand there is an increase in the cephalic flexure. Five gill invaginations are usually visible, the two posterior ones being sometimes indistinct. There are distinct nasal pits. The roof of the medulla is becoming thin and transparent. From twenty-five to thirty mesoblastic somites are apparent in surface views. The tail undergoes a decided increase in size, and is slightly flexed ventrally. The front limb anlagen appear during the latter part of this stage.

The lateral vascular bands are not yet differentiated into veins, but during the latter part of this stage some small veins have been observed, in living material, extending vertically from the vascular bands for a short distance above them (fig. 192). No



Figs. 192 to 197 Development of the vitelline veins of *Cryptobranchus allegheniensis*. All the figures are from living material and, with the exception of the veins in figure 192, are drawn with the aid of a camera. $\times 5$.

Fig. 192 Stage 20. The stippled area indicates the lateral vascular band. The right and left sides of the embryo are practically alike.

Figs. 193 and 194 Right and left views of an embryo in Stage 22.

Fig. 195 Lateral, slightly ventral, view of an embryo nearly ready to hatch.

Fig. 196 Ventral view of an embryo nearly ready to hatch.

Fig. 197 Ventral view of a larva about ten days after hatching.

movement of the blood has been observed in this stage. The embryos are ciliated, and undergo rotation, much as in the preceding stage. Muscular movements do not ordinarily occur, but have been observed when the embryos were placed in fixing fluids.

Stage 21: (figs. 191 and 252 to 255). This stage is reached about four days later than the beginning of Stage 20. It is characterized by the presence of three budding external gills, and small front limb rudiments. The tail is longer, and has a more decided ventral flexure, than in the preceding stage.

The dorsal surface of the embryo is now for the first time slightly pigmented. The pigmentation begins in that portion of the ectoderm overlying the nervous system, and gradually extends downwards over the sides of the body. During this and the following stages (McGregor '97), the pigment cells in the region of the mesoblastic somites are grouped metamerically.

Numerous veins now branch off dorsally from the lateral vascular bands. In the bands themselves, the two main trunks of the omphalo-mesenteric or vitelline veins, one on each side of the yolk sac, are being differentiated, but they rarely become complete before the next stage. In general the differentiation of the vitelline veins has gone further on the side of the body that happens to be uppermost. During the latter part of this stage the heart is pulsating regularly, with about twenty-five to forty beats per minute; the blood sometimes surges back and forth in the principal veins overlying the yolk sac, with a slight excess in the forward movement, but the vitelline circulation is not yet completely established.

The distribution of cilia remains much the same as in the preceding two stages, but the ciliary currents move more uniformly toward the posterior end of the body (fig. 191).

Spontaneous muscular movements, consisting chiefly of a bending of the body laterally into a *U* shape, now occur, but the embryo is as yet unable to turn over.

Stage 22: (figs. 193, 194 and 256 to 259). This stage begins about five days later than Stage 21; its most distinctive characteristic is that the external gills have rudimentary branches and

are pink with blood. The tail is rapidly increasing in size and beginning to straighten out. The front limb rudiments now take the form of conspicuous outstanding lobes, the distal ends not yet divided into digits. Toward the close of this stage the rudiments of the hind limbs appear. The entire dorsal surface of the body is well sprinkled with pigment cells; the eyes are becoming deeply pigmented. The lateral line system is well developed. During this stage occurs the rapid formation of the gular folds.

The lateral vascular bands no longer appear as such, but on their site are differentiated the two main trunks of the vitelline veins (figs. 193 and 194). As compared with the earliest position of the lateral vascular bands, the vitelline veins lie considerably nearer the ventral surface of the yolk sac. While the vitelline system of veins is primarily a paired one, almost from the beginning one side is usually found better developed than the other. The heart now contracts at the rate of about forty to sixty beats per minute, and the blood pulsates regularly through the vitelline veins.

The cilia are especially well developed on the external gills; here the ciliary currents are strongest. Spontaneous muscular movements now occur at frequent intervals. The movements consist of jerking the head from side to side; wriggling; reversal of the laterally curved position of the body by turning over; swimming movements by means of which the embryo butts against the envelope; and swimming in a circle. The functional value of these movements seems to be to afford exercise for the developing muscles. Embryos removed from the capsules at this stage make practically the same movements; they are unable to progress in a straight line and are incapable of prolonged swimming movements.

During the later stages of development before hatching, the water in which the embryos are kept has a pronounced 'fishy' odor.

Stage 23: (figs. 195, 196, 260 and 261). The limits of this stage are fixed to include the time of hatching. In a given lot of embryos hatching does not take place all at once, but extends

over a period of about a week, with a corresponding variation in the degree of development of different individuals at the time of escape from the envelopes. On the average, hatching occurs about two weeks later than the beginning of Stage 22, and about six weeks after fertilization. Previous to the hatching period, the envelopes become much softened and considerably enlarged by the absorption of water, making room for the growing embryo. The latter usually escapes by pushing ('worming') its way through the envelope, leaving a small round hole; in some cases it bursts the envelope by means of wriggling movements.

The newly hatched larva measures about 23 to 25 mm. in length. Very noticeable is the retention of a large yolk sac with conspicuous bright-red vitelline veins; the bushy external gills are pink with blood. In proportion to size of body, the tail is much larger than in the adult. The dorsal surface of the body and the sides of the tail are well pigmented, but in general the larva of *Cryptobranchus*, like that of *Necturus*, is pale as compared with amphibian larvae that develop from a pigmented egg exposed to the light. The ventral surface is lacking in pigment, leaving the abdominal region yellow from the presence of yolk, and the throat region transparent. The heart can be readily observed without dissection. The anterior limb rudiment is provided with two digits. In most specimens the body somites are plainly visible, but they do not show well in the photographs. On account of its large size, graceful outlines and bright colors, the newly-hatched larva is a striking and beautiful object.

In the resting position, the larva lies on its side, turning occasionally from one side to the other. The newly hatched larva is able to swim rapidly in a straight line for a short distance, using the tail as a propeller. The larvae avoid the light, and are positively rheotactic.

The vitelline veins (figs. 195, 196 and 261) have shifted further toward the mid-ventral line; on one side of the body these veins are well developed, on the other side they show arrest of development with signs of atrophy. The heart now beats about sixty to seventy times per minute.

Aëration of the blood is afforded, not only by the external gills, but by the capillaries lying close to the surface over all the body. On account of its great exposed surface, the tail may be of especial importance as a respiratory organ.

As in the preceding stage, cilia persist over the entire surface of the body, and the ciliary currents are strongest in the vicinity of the gills.

B. Larval development, and the metamorphosis

The changes in the form of the body, and the gradual increase in size, during the first year of larval development, are shown by the photographs (figs. 262 to 267).

Year-old larvae reared in the laboratory reach a length of about 5 to 7 cm.; three two-year-old specimens reared in the laboratory measured respectively, after preservation in alcohol, 7 cm., 8 cm. and 9.5 cm. Near the close of the second summer these latter specimens lost their external gills. The few specimens with external gills taken in August from their natural environment (they were found under small flat stones in shallow water) measured as follows: 6.4 cm., 6.8 cm., 7.0 cm., 7.3 cm., 7.7 cm., 12.0 cm., 12.3 cm. It will be noticed that these specimens sort into two lots, one lot containing those with body lengths ranging between 6 and 8 cm., the other lot containing specimens approximately 12 cm. in length. Though this data is rather meager, the rather considerable gap between the two lots suggests that we are dealing with larvae of the first and second summers, respectively. In comparing the larger larvae taken from their natural environment with the two-year-old specimens reared in the laboratory, allowance must be made for the fact that the latter were measured after being shrunk by preservation in alcohol; moreover the body form of the laboratory specimens seems to be shorter and stouter than the normal. Specimens with a body length of 14 cm. and more, taken from their natural environment, have invariably lost their external gills. The combined evidence from specimens reared in the laboratory and

those taken from their natural habitat indicates that the metamorphosis occurs at the end of the second year.

At the time of hatching, the embryo retains a supply of yolk sufficient to last it for several months; the mouth is still quite ventrally situated. So far as its method of nutrition is concerned, during this period the young *Cryptobranchus* is an embryo rather than a larva. Gradually the yolk disappears, and the mouth assumes a terminal position. Specimens reared in the laboratory begin to take food about two to four months after hatching; they must be fed individually with bits of scraped beef. No notice is ordinarily taken of the food unless it is moved about immediately in front of the animal and preferably a little to one side of the mouth. Some of the specimens take food more readily, and grow more rapidly, than others. One lot of larvae, reared in the laboratory, ate young frog tadpoles. A 12 cm. specimen taken from its natural habitat ate a large *Corydalis* larva; another newly captured 12 cm. specimen regurgitated a partly digested 6 cm. larva of its own kind.

During the first month after hatching, the vitelline veins of one side of the yolk sac degenerate, while those of the other side shift to a more nearly median position (fig. 197). Degeneration of the right or the left vitelline vein takes place in about an equal number of cases. It has already been noted that in those stages when the embryo lies continuously on one side the vitelline veins are best developed on the uppermost side; furthermore that the embryo falls on the right or the left side in about an equal number of cases. The facts strongly suggest that the position of the embryo during the period when the vitelline veins are developing is the factor that determines on which side the vitelline vein shall persist; but since making these observations I have had no opportunity to put the matter to a rigid test.

With the reduction of the yolk sac, the vitelline circulation suffers a corresponding diminution in extent; during the late stages of this process, through the increasing thickness and opacity of the ventral body wall the vitelline veins are somewhat obscured.

In the free-swimming larva, the heart beats more rapidly than was the case during embryonic development.

Within a week or two after hatching, the rapid growth of the front limb rudiments enables the larva to support itself in the normal position of the adult. One month after hatching, the front limbs have increased decidedly in length and possess the full number of digits (four). The form and position of the front limbs adapt them for use as paddles; by means of a simultaneous backward stroke they aid the larva in getting a quick start for swimming. The posterior limbs develop more slowly; at this time they are relatively short and as a rule possess but three digits, though in some cases the full number (five) are present. Ten weeks after hatching in all cases both pairs of limbs possess the full number of digits and are used in walking in the same manner as in the adult. The limbs are broad and flat, and in swimming at a moderate rate of speed are used as paddles. After the sixth month of larval development the posterior limbs surpass the anterior in size and strength. In the two-year-old specimens reared in the laboratory the limbs appeared weak and poorly developed as compared with newly captured specimens of the same age.

As in the adult, the tail is the principal organ of locomotion during rapid swimming. Up to five or six months after hatching the tail remains much larger in proportion to body-size than in the adult. In the year-old larva the tail is proportionally much smaller than in specimens ten weeks after hatching. In the two-year-old specimens reared in the laboratory the tail is smaller than in newly-captured specimens of the same age.

One month after hatching, pigmentation is greatly advanced and extends over the external gills; when viewed from above the larva is now nearly black. The ventral surface remains white and nearly transparent in the throat region, yellow in the region of the yolk sac. Six months after hatching, the dorsal surface shows large dark spots of unusually dense pigment, which are characteristic of all the later stages; the ventral surface is slightly pigmented. In the year-old larva, the dorsal surface is still nearly black, but with a few scattering inconspicuous yellow spots; the abdomen is grayish and the throat region almost

white. In the two-year-old larva the general color effect is not so dark; the larva is taking on the variegated color pattern of the post-larval and adult stages.

In the month-old larva, shedding of the cuticle was observed for the first time. From this time on, the water of the aquarium becomes almost cloudy with detached portions of epidermis.

Soon after the hatching period, cilia disappear from the general surface of the body, but persist on the gills where a strong eddy current of water is produced. The gills remain ciliated until at least six months after the hatching period; at this point observations were necessarily discontinued.

As early as five months after hatching and frequently thereafter, larvae have been observed to come to the surface for air and to give up large bubbles of air from the mouth, indicating that the lungs are functional.

During the first year of larval life, the ventral portions of the three gill arches bearing the external gills are expanded into leaf-like plates, partly covered by the opercular lateral portions of the gular fold. At this time there are three gill openings. Toward the end of the first year the median portion of the gular fold disappears, but the lateral portion extends as a small opercular fold above the root of the anterior external gill. During the second summer the opercular fold extends dorsally far enough to cover the bases of all three external gills. With the loss of the external gills the opercular flap persists and partly roofs over a shallow cavity containing the leaf-like plates previously mentioned.

C. Post-larval stages

Sexual maturity is attained with a length of at least 30 cm. for the male, and 35 cm. for the female. During August, specimens 14 to 20 cm. in length have been very rarely taken, while specimens measuring from 20 cm. upwards are very plentiful; this suggests that the young ordinarily reach a length of at least 20 cm. at the end of the third year, and that they do not ordinarily become sexually mature until the end of the fourth year.

The immature post-larval stages resemble in coloration the young adults described in Part I. Aside from the loss of the external gills, the most conspicuous changes as compared with the larvae are a slight progressive dorso-ventral flattening of the body, and the gradual development of the folds of the skin which are so prominent in adult and especially in very large and presumably old specimens. A transverse slit-like spiracular opening is bordered anteriorly by the small opercular flap and posteriorly by a similar but still smaller fold of skin. The spiracle leads to a small cavity containing a single persistent gill opening bordered by two leaf-like plates; the other gill-lamella is greatly reduced or has disappeared.

D. Summary

The vitelline membrane is shed about the time of the closure of the neural folds.

Shortly after the closure of the neural folds, the entire surface of the embryo becomes ciliated. The beat of the cilia is in general toward the posterior end of the body; currents of water are produced which are subservient to respiration. After the appearance of the external gills, ciliary currents are especially strong in their immediate vicinity. Soon after the hatching period, the cilia disappear except on the external gills; here they persist until at least six months later.

Soon after the closure of the neural folds, the embryo falls on one side, where it lies until, in a much later stage, it is able to turn over through muscular activity. The embryo falls on the right or the left side in about an equal number of cases.

Rotation of the embryo, due to the beating of the cilia, commences before the embryo has fallen on its side but is more pronounced afterward. In most cases rotation proceeds in a clockwise direction when an embryo is lying on its right side, in an anti-clockwise direction when it is lying on its left side. Rotation may be of service in preventing adhesion of the embryo to the capsule.

The vitelline veins develop as paired structures along the sides of the yolk sac, and shift gradually toward the ventral

median line. The vitelline veins develop more rapidly on that side of the yolk sac which happens to be uppermost. Soon after the hatching period, the vitelline veins of one side degenerate, while those of the other side reach their fullest development; the veins of the right or the left side persist in about an equal number of cases. Probably the position of the embryo during the stages when it lies continuously on one side is the factor that determines which set of vitelline veins shall gain the ascendancy.

The newly hatched larva retains a supply of yolk sufficient to last it from two to four months.

The tail of the early larva is proportionally much larger than in the adult.

Pulmonary respiration is established about five months after the hatching period.

The metamorphosis takes place at the end of the second year.

Sexual maturity is attained, probably at the end of the fourth year, with a body length of at least 30 cm. for the male and 35 cm. for the female.

X. TIME RECORD

For the characteristics of the different stages reference is made to the text and illustrations, especially the photographs. For methods used in obtaining the time record, see the introduction (Section VI). Table 1, page 534.

XI. ABNORMALITIES

The present section deals primarily with abnormalities found in embryos taken from their natural environment, or kept under conditions as nearly normal as possible.

1. *Large yolk plug*

In the early stages of development the most common abnormality is the presence of an unusually large yolk plug. Examples are shown in figures 198 and 199, though these are far from representing extreme cases. In well-marked examples the blastopore forms a complete circle only a little below the equator, and

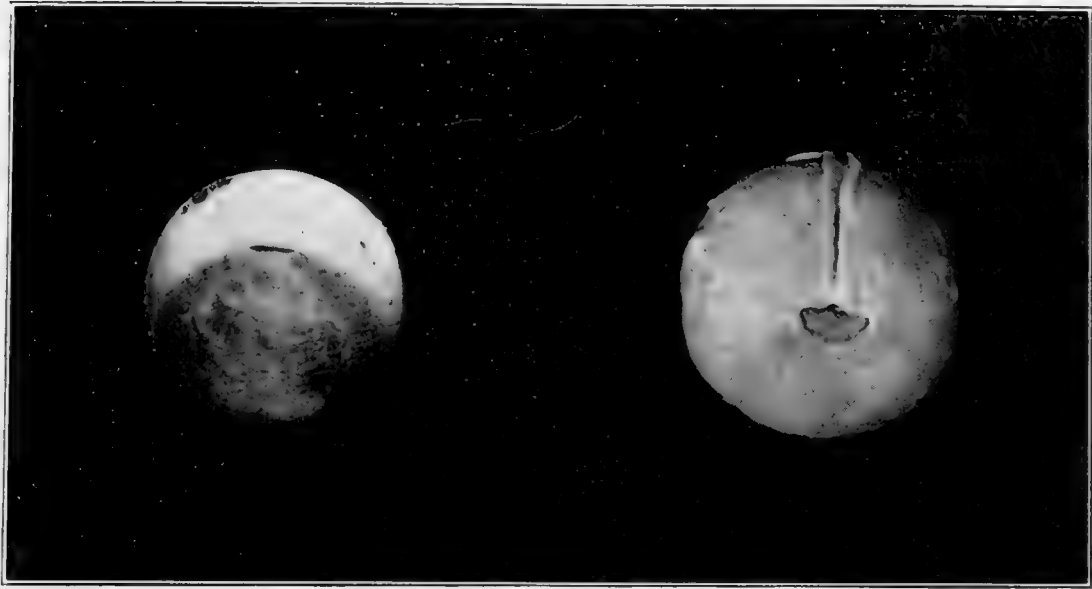
TABLE 1

STAGE	TIME AFTER FERTILIZATION	INTERVAL
1	24 hours (18 to 28)	
2	30 hours (24 to 36)	6 hours (5 to 7)
3	35 hours (29 to 44)	5 hours (4 to 6)
4	39 hours	4 hours (3 to 5)
5	43 hours	4 hours
6	47 hours	4 hours
7	51 hours	4 hours
8	63 hours	12 hours
9	3½ days	19 hours (14 to 24)
10	5 days	36 hours (24 to 48)
11	7 days (6 to 8)	48 hours
12	10 days	72 hours
13	11½ days	36 hours
14	12½ days	24 hours
15	13¼ days	18 hours
16	14 days	18 hours
17	15 days	24 hours
18	16 days	24 hours
19	17 days	24 hours
20	19 days	48 hours
21	23 days	4 days
22	4 weeks	5 days
23	6 weeks	2 weeks

Metamorphosis: Two years after fertilization.

a large yolk plug persists long after the closure of the neural folds; such embryos usually produce normal larvae, though spina bifida is an occasional result. In extreme cases the blastopore may form entirely above the equator; such embryos die before reaching the larval stage.

This abnormality appears most frequently in eggs that have been kept in jars of water during warm weather, and especially in material that has been shipped long distances. Probably it may be brought about by a variety of unfavorable conditions: heat, lack of oxygen, mechanical agitation, and injurious substances (e.g., cinders) in the water. It is readily produced by treatment with sodium chlorid; very marked cases of spina bifida may result.



198

199

Figs. 198 Postero-dorsal view of a gastrula of *Cryptobranchus allegheniensis* with an unusually large yolk plug. The blastopore extends around the egg as a complete circle, but only the dorsal portion shows distinctly in the photograph. From preserved material. $\times 4$.

Fig. 199 Posterior view of an embryo shortly before the closure of the neural folds, showing persistent yolk plug. Photographed from preserved material. $\times 4$.

2. *Exovate abnormality*

A rather common abnormality originating at the time of the formation of the neural groove may be called the exovate abnormality. A small protuberance on the site of the closed fenestra takes the form of a spherical exovate; in some cases this reaches a diameter about half as great as that of the egg. In most cases the extra-ovate remains connected with the egg by a very narrow stalk; the malformation is entirely extra-embryonic, the protruded yolk is gradually absorbed and the egg produces a normal larva. In other cases the protuberance takes the form of a dome-like swelling and increases in size until the egg collapses.

3. *A double embryo*

On September 27, 1906, I found a nest containing embryos in an advanced stage of development, and among them the double

embryo shown in figure 200. The total bulk of this double embryo is about equal to that of an ordinary single embryo from the same spawning. That the occurrence of such a monstrosity in this species is exceedingly rare in nature is shown by the fact that during the past seven years I have collected many thousands of embryos from nests, yet in no other instance have I found an abnormality even approaching the one under consideration.



Fig. 200 Double embryo of *Cryptobranchus alleghehniensis*. Photographed after preservation in formalin. $\times 4$.

The precise manner in which the double embryo originated is problematical. Single capsules sometimes contain two eggs in close contact; but the small size of the double embryo precludes the possibility that it was formed by the union of two such eggs. Through treatment with sodium chloride I have produced embryos in which the brain differentiated without closure of the neural folds, but no true double embryos were obtained by this means; moreover in nature it is improbable that unusual chemical influences should affect a single egg.

It is more likely that the abnormality was brought about by mechanical means. A partial separation of the first two blasto-

meres (e.g., through the egg becoming lodged in a crevice) might in some cases result in the formation of a double embryo (Spermann '01 to '03). But owing perhaps to the heavily yolk-laden character of the egg, and the slowness with which the first furrow cuts its way through the yolk, my attempts to produce a double embryo in this manner have failed. When a fine silk thread or a hair was tied about the egg in the two-cell stage so as to constrict it in the plane of the first cleavage furrow, the egg usually burst before reaching the gastrula stage. In a single case the egg lived until after the formation of the neural folds, but developed a single embryo with its principal axis at right angles to the constricting cord.

A more probable explanation is that in the two-cell stage the egg became inverted and remained for some time in this position subject to the rearrangement of contents through the disturbing influence of gravity acting in a direction opposite to the normal (Schultze '95). But the whole question is complicated by the more fundamental problem of the determination of the median plane of the embryo. My experiments, to be described in a later paper, show that in *Cryptobranchus* the first cleavage furrow tends to form at right angles to the direction of entrance of the sperm (as in *Triton*, but not as in the frog where the first cleavage tends to coincide in direction with the path of the sperm). If in *Cryptobranchus*, as in the frog, the entrance path of the sperm lies approximately in the median plane of the embryo, then the conditions necessary for the production of a double embryo by the separation of the first two blastomeres must be of very exceptional occurrence.

4. Spiral-tailed monsters

In the fall of 1910, some embryos shipped by express to the University of Wisconsin and reared there in city water, acquired, in about 8 per cent of their number, the abnormality shown in figures 201 and 202. From the condition of the tail in late larval stages, specimens affected with this malformation may be designated as 'spiral-tailed monsters.' At first this peculiarity seemed

merely the persistence of an embryonic condition; for in early stages the tail is always strongly flexed and in these particular cases it failed to straighten out. But as development progressed the tail became twisted into a pronounced spiral. The illustrations show the extreme condition; cases occur forming a series intermediate between this and the normal. In some cases the back is arched or humped. So far as can be judged from surface views,

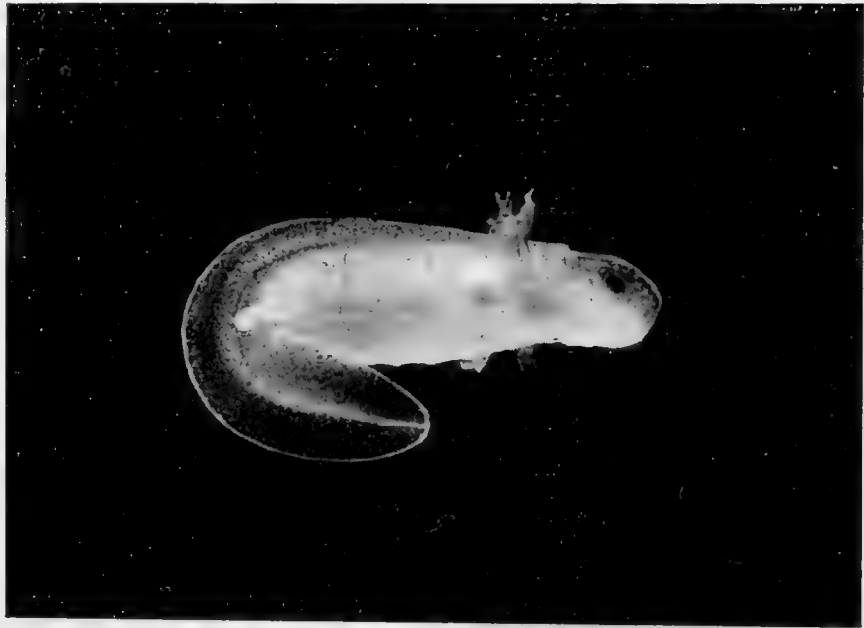


Fig. 201 Spiral-tailed monster of *Cryptobranchus alleghehiensis* at the time of hatching. Photographed from the living embryo anaesthetized with chlore-tone. $\times 3$.

the entire abnormality seems to be brought about by the constricting effect of a band of tissue lying in the ventral median line.

As a result of this malformation, the larva is compelled to lie on one side, and can swim only in a grotesque fashion, with backward circling movements. Affected larvae take no food, and die shortly after using up their supply of yolk.

An analysis of the water was secured but the subject has not yet been further investigated. Stockard ('06, p. 119) noted the occurrence of a similar abnormality in *Fundulus* embryos.

5. Critical periods in the life history

In closing this section it remains to note that there are three stages in the life history characterized by unusual mortality—nature's examinations occur in these stages. These critical periods of development are:



Fig. 202 Spiral-tailed monster of *Cryptobranchus alleghehiensis* two months after hatching. Photographed from the living larva anaesthetized with chlore-tone. $\times 3$.

(a) *The late blastula.* Many eggs seem unable to form a gastrula.

(b) *The hatching period.* Many embryos seem to lack the strength necessary to escape from the gelatinuous envelope, and die in the capsule.

(c) *The period of change in the method of nutrition.* After exhausting their supply of yolk, many larvae refuse to take food, or seem unable to set up digestive processes, and die of starvation.

XII. PHYLOGENY

We are here concerned with (a) the origin of the amphibia in general, (b) the origin of the urodeles, and (c) the interrelationships of the urodeles with special reference to *Cryptobranchus*. The evidence may be classified as (a) anatomical, (b) paleontological, and (c) embryological. In the following survey, no attempt is made to keep these lines of evidence strictly separate; for paleontology is simply an extension of comparative anatomy to fossil forms, with the added element of sequence in time; while the development during the late embryonic and larval stages gives the key to many adult structures that could not otherwise be homologized.

It is not within the scope of this paper to enter extensively into anatomical and paleontological questions; but as a check on possible generalizations derived from the study of amphibian embryology the writer has devoted considerable time to a study of the paleontological material in the American Museum of Natural History, and has endeavored to acquire some degree of familiarity with the results of modern research in this field. In this work he has been greatly aided by Dr. W. K. Gregory, who has in progress a detailed review of the origin of the amphibia (see abstracts in *Science*, Gregory '11 a, '11 b and '12).

The tetrapoda form a coherent group. The gap between this group and the fishes is one of the weak places in vertebrate phylogeny, and it is here that the idea of continuity in the descent of the higher vertebrates has been most often attacked; some have maintained a diphyletic origin for the tetrapods and fishes.

The amphibia undoubtedly include the most primitive known tetrapods. It is on this account that the amphibia possess a peculiar interest from a phylogenetic point of view; the problem of the origin of the amphibia is the problem of the origin of the tetrapods in general.

The fundamental unity of the gnathostome type leads us to look among the true fishes for the nearest living or fossil representatives of the ancestral stock of the amphibia. For in fishes and tetrapods the following features, as well as many others,

are homologous: the chief divisions of the brain; the cranial nerves; the eye muscles and their innervation; the chondrocranium arising from trabeculae and parachordals, and including olfactory, optic and auditory capsules; the visceral arches; and the essential structures of the circulatory system. If we compare only the amphibia with the fishes the range of resemblances becomes still greater; all the important structures are in essential agreement except those concerned with the outer halves of the paired extremities.

The argument for a diphyletic origin of the pisces and amphibia is based upon (a) the lack of homology in certain elements of the skull, and (b) the difficulty that is experienced in deriving the amphibian limb from the fin of any known fish. But the resemblances in the skull bones become very close when we consider fossil forms, and the trend of increasing knowledge is in the direction of more complete homology rather than the reverse. Moreover we find difficulties almost as great in homologizing cranial elements in different fishes; for these structures are plastic and exposed to environmental influences, and with the radical change from an aquatic to a terrestrial habitat we should expect them to be profoundly influenced. The endoskeleton of the paired appendages presents us with a problem of greater difficulty but here, too, we have to deal with structures that we should expect to be greatly modified in connection with the change of habitat. In view of the wide range of resemblances in important structures one is hardly inclined to consider seriously the idea of a diphyletic origin for the fishes and amphibia.

The amphibians must have descended from some fish having scales with the potentiality of fusing into bony plates. The dermal bones forming the roof of the skull must have been arranged in pairs on each side of a median suture; for this is the condition found in the most primitive known amphibians (e.g., *Branchiosaurus*). The ancestral form must be sought in some fish having the endoskeleton of the paired fins widely protruded from the body, and with pectoral and pelvic members similar. Such fins functioned primitively as paddles; with the adoption of a terrestrial method of locomotion, by creeping or crawling on a

more or less solid substratum, the endoskeletal elements of the limbs become greatly strengthened and progressively longer in order to lift the body from the ground. Such a progressive elongation of the limb bones, particularly the proximal elements, may be traced in both fossil fishes and amphibians; in the latter the pelvic girdle is also found becoming definitely articulated with the axial skeleton. The ancestral form must have been short-bodied; for in many groups of animals a progressive elongation of the body, culminating in eel-like forms, is found to accompany a degenerate structure scarcely capable of giving rise to higher forms (Gregory '07, Appendix I).

The lack of scales with the potentiality of fusing into bony plates is alone sufficient to exclude the elasmobranchs from the immediate ancestry. For affinities ancestral to the amphibia most authors have looked to the crossopterygii or the dipnoi. Both have dermal bones, and both fulfil the requirement regarding fins with widely protruded basal lobes and with endoskeletal elements from which the framework of true limbs might be derived.

At first sight the dipnoi seem best to bridge the gap between fishes and amphibia. For the lung-fishes have survived by virtue of an approach to the tetrapod type, enabling them to exist during periods of drought. But various considerations derived from the study of paleontology and comparative anatomy make it probable that these terrestrial adaptations were independently acquired, and that the dipnoi were already too highly specialized in other respects to give rise to the amphibia. The mosaic of small bones forming the greater part of the roof of the skull, particularly in the fossil representatives of this group (e.g., *Dipterus*), and the usual occurrence of one to several large median elements in this region, make it difficult or impossible to homologize the dermal bones of the skull with those of amphibia. The characteristic dentition is far removed from that of the amphibia. Marginal teeth, with exceptions in the cases of some very early forms (e.g., *Phaneropleuron*), are lacking; in the later forms the loss of maxillae, premaxillae and nasals shows a progressive tendency toward degeneration in these regions. The concen-

tration of the teeth into tritoral clusters in the roof and floor of the mouth is not in itself an unfavorable feature, for vomerine teeth occur in the amphibia; but in all except certain very early fossil forms (e.g., *Uronemus*) the fusion of these teeth into large dental plates with grinding ridges has gone too far to give rise to the condition found in the amphibia. So far as the paleontological and anatomical evidence is concerned, the known facts tend to exclude the dipnoi from the direct ancestry amphibia, of the yet do not wholly preclude the possibility that future discoveries may supply us with more favorable material amongst early fossil forms. In so far as the terrestrial adaptations of the dipnoi resemble those of the amphibia, the case may be one of parallelism or convergence; their more fundamental resemblances indicate that they are not very far removed from a common ancestry.

Turning to the crossopterygii we find more favorable anatomical and paleontological grounds for comparison with the amphibia. The dermal elements forming the roof of the skull occur in paired series; a large number of cranial bones may be definitely homologized with those of the amphibia (see especially Baur '96; Moodie '08 a; for materials for further comparison see Goodrich '09, and Zittel '11). It is difficult to believe that identical relations in so many bones could have been independently evolved. With regard to the fins, we find examples of a bifurcated type of endoskeleton that makes a more favorable starting-point for a tetrapod limb than the archipterygial type of the dipnoi; e.g., see fig. 203 for the pectoral fin of *Sauripterus*, and Goodrich '09, p. 275, fig. 244, for the pelvic fin of *Eusthenopteron*; the pectoral fin of *Eusthenopteron* (Goodrich '09, p. 282, fig. 252) is not quite so favorable. But both of these forms belong to the *Rhizodontidae*, whose skull is not so favorable for comparison with the amphibia as the skull of some other crossopterygians; in no one form do we find all the conditions ideal for the derivation of the tetrapod type. The occurrence in *Polypterus* of two kinds of ribs, both the ventral or pleural ribs characteristic of the teleostomi and dipnoi, and the dorsal ribs characteristic of elasmobranchs and tetrapods, is a point emphasized by Baur

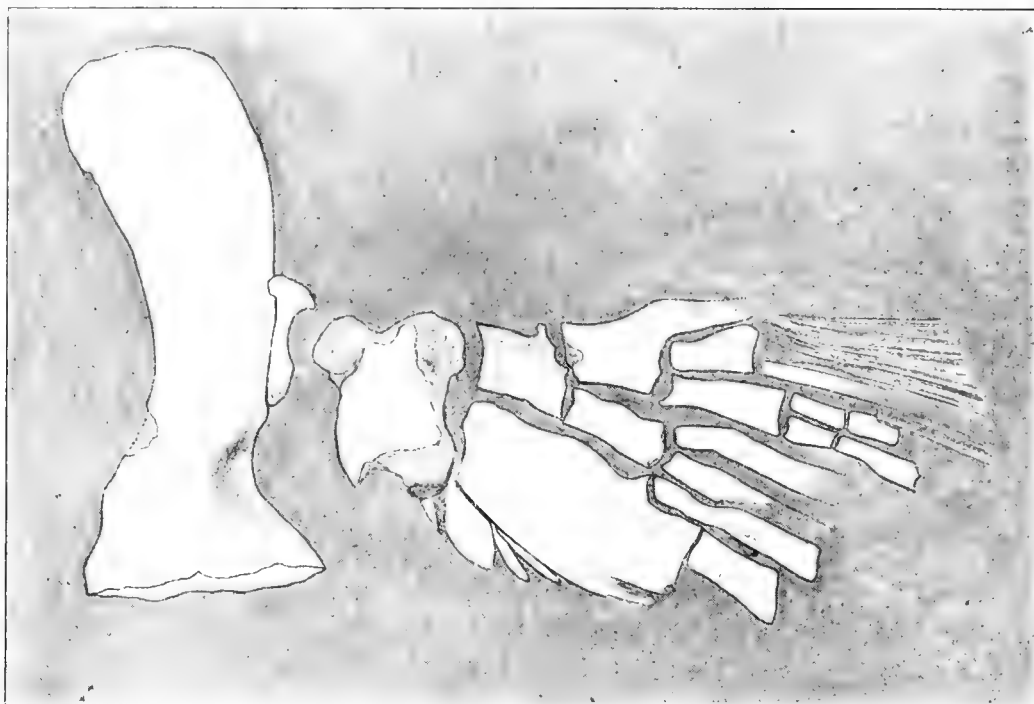


Fig. 203 Endoskeleton of the pectoral fin of *Sauripterus taylori* Hall, a crossopterygian from the upper Devonian. One-third natural size, linear reduction. From a drawing by Dr. L. Hussakoff of the American Museum of Natural History.

('96) as favoring a crossopterygian rather than a dipnoan ancestry for the amphibia.

It is upon embryological grounds that the strongest case has been made out for the derivation of the amphibia from the dipnoi; the known facts of development indicate the common origin and later separation of these two groups. In so far as this view is based upon a study of the early stages, the evidence may be dismissed with the remark that the early development of the crossopterygian *Polypterus* (Kerr '07 a) resembles that of the anura and the urodeles quite as much as does the early development of the dipnoi (Semon '00 and '01; Budgett '01; Kerr '00, '01 and '09); furthermore that these early stages are of very little value in connecting up the great groups of vertebrates. But some marked resemblances between dipnoi and amphibia in the later stages of development cannot be disregarded. Kellicott ('05, a and b), on the basis of a detailed study of the circulatory system of *Ceratodus*, came to the conclusion: "The resemblance in the vascular and respiratory systems between *Ceratodus*, the

most primitive of the dipnoi, and the amphibia, especially the urodeles, are numerous and important, and cannot be explained as parallelisms." In this connection it is important to compare the development of the circulatory system of *Polypterus*. From the account given by Kerr ('07), it appears that in *Polypterus* the vascular system, particularly because of the presence of only a single pair of aortic arches, is decidedly less amphibian in character than that of *Ceratodus*.

In the present state of our knowledge it is impossible to reach an unqualified decision of the question under consideration. In weighing the evidence one should not forget that in numerous cases where anatomical and embryological evidence have come into conflict in deciding questions of the phylogenetic relationships of the larger groups of animals, it is the embryological evidence that has had to give way, and that recent anatomical evidence has to give place to paleontological. Whatever light may be shed by future discoveries on the question of the derivation of the amphibia from the crossopterygii or the dipnoi, it is clear that the point of origin is not far from either stock; in other words, that the three lines of descent have separated from a common stem at no very great intervals.

Concerning the immediate ancestry of the living amphibia we have detailed evidence only in the case of one group, which fortunately for our purpose is the caudata or urodela. According to Moodie ('08 b) the urodeles are descended from the branchiosauria, a group of primitive extinct amphibia from the carboniferous and Permian. These are small, short-tailed amphibians with broad heads. The skull is slightly more complex than in the urodeles, and there is a dermal exoskeleton consisting of rows of thin semi-cycloid scales, especially on the flanks and under side of the body. External gills are present in the larvae. The view that these forms are ancestral to the urodeles is based on a detailed comparison of the structure of the skull, the structure and form of the vertebrae and the ribs, the number of digits, the arrangement of the phalangeal elements, the character of the pectoral and pelvic girdles, the distribution of the lateral line system, the structure and form of the long bones, and finally

the shape of the body. According to Moodie the caudata are degenerate branchiosaurians and the changes which have taken place in the exoskeleton are mostly brought about by the loss of certain parts. The urodele skull is especially degenerate in the occipital and temporal regions; it may be derived from the skull of *Branchiosaurus* (for which see Credner '81 to '90) by the loss of the dermoccipitals (supraoccipitals of Moodie '08; postparietals of Zittel '11), supratemporals (Zittel '11), postfrontals, postorbitals, sclerotics, epiotics (tabulares of Zittel '11), jugals and quadratojugals. The urodele skull has also in many cases become narrow in connection with a general elongation of the body—a degenerate feature. Hand in hand with the loss of dermal elements in the skull has gone the loss of the exoskeleton of the body.

The paleontological history of the apoda is unknown; but existing members of the group are in certain respects more primitive than the urodeles and more nearly allied to the stegocephali. Thus the hyoid and branchial apparatus is more primitive than that of any other recent amphibia; dermal scales are present which are probably homologous with those of the stegocephali. As in the urodeles, the skull shows degeneration in the loss of certain bones; but the epiotics (tabulares) are retained, and occasionally the postfrontals and the lacrimals. A second row of teeth is sometimes present on the mandibles. Aside from some very degenerate features, in other respects the apoda are highly specialized, indicating, as in the case of the anura, a line of descent separate from the urodeles. Yet there are some very suggestive resemblances. Attention has already been directed (Smith '12) to the marked similarity in structure of the egg envelopes of *Ichthyophis* to those of *Cryptobranchus* and *Amphiuma*, and to the likeness in the brooding habits; but in *Ichthyophis* the female protects the eggs, in *Cryptobranchus* the male. In their embryological development the apoda show many points of similarity to the reptiles.

With this background, we now come to the question of the interrelationships of the urodeles. In particular we are concerned with the phylogenetic position of the aquatic urodeles (the peren-

nibranchs and the derotremes²) as related to the land-living salamanders.

The most prevalent view has been that the aquatic urodeles are the most primitive. Parker and Haswell ('97, vol. 2, p. 291) have said: "The perennibranchiate urodeles are undoubtedly the lowest of existing amphibia; they lead up, through such forms as *Amphiuma*, with persistent gill slits but deciduous gills, to the land salamanders, in which a purely terrestrial form is assumed." In a group standing between fishes and the typically terrestrial vertebrates, it is natural to regard the aquatic forms as transitional to the terrestrial. In fishes there are usually five branchial arches, and the gill slits remain open throughout life. The land-living salamanders have in the adult state only two branchial arches (Parker '77; Wiedersheim '77; Cope '89), and the gill slits are open only to the end of larval life. In the aquatic urodeles there are usually three or four branchial arches (as in the larvae of the terrestrial forms), and the gill slits usually remain open throughout life—conditions intermediate between fishes and salamanders. In view of the occurrence of external gills in all larval forms, the persistence of such gills in the perennibranchs might, on the recapitulation theory, be regarded as a primitive character.

In the opinion of various authors, the above interpretation represents a short-sighted view of the matter. Boas ('81) was the first to assert that the perennibranchs are larvae that have lost the ability to transform; this conclusion was reached as a result of a comparative study of the circulation. Cope ('85) described the retrograde metamorphosis of *Siren*, and concluded that the present *Sirens* are descendants of a terrestrial type. Gadow ('01, p. 66) suggested a terrestrial ancestry for the urodeles. Kingsley ('01) says: "The salamandrina form the central urodele stem, and the perennibranchs and derotremes have been derived from this stem by degeneration and the retention of larval characters." Kingsbury ('05), on the basis of a compar-

² The classification of Stannius ('56) is here followed, as it seems best adapted for the purposes of this discussion.

ative study of the cranial elements, came to the tentative conclusion that *Necturus* is a permanent larva. The generalization formulated by Boas has recently been reiterated and expanded by Versluys ('09); briefly stated, his views are as follows:

The great resemblance of the perennibranchs to salamander larvae is only a consequence of the fact that the first are also larvae, but larvae which no longer come to full development as their ancestors did; the metamorphosis is imperfectly undergone or wholly omitted. Nevertheless these larvae become sexually mature, as in neoteny. In the course of time, in adaptation to their aquatic habitat, they have become degenerate in many respects. The derotremes are salamanders that have become fixed in the transitional stage or metamorphosis. Thus the aquatic urodeles have a terrestrial ancestry; they are forms which have reverted to an aquatic mode of life.

The probable course of events giving rise to the perennibranchs may be described as follows: While the mature salamanders are constructed after the fashion of a land animal, their larvae live in water and in the course of time have become more and more adapted to aquatic life. They have extended their larval organization and thus increased the difference, which must be overcome by metamorphosis, between the larva and the grown-up animal. In time some organs show arrested development or degeneration of such a sort that the larvae can no longer develop into land-living salamanders; they remain life-long water dwellers. The condition is one of fixed neoteny (Boas '96). According to this view the perennibranchs are disconnected from one another and have evolved as neotenic larvae from different salamanders.

Confirmatory evidence for this view comes from the studies of Emerson ('05) on *Typhlomolge*. In some details of its structure this animal shows a remarkable similarity to the larvae of *Spelerpes ruber*. Probably *Typhlomolge* has been derived from the neotenic larva of some salamander of the family (Plethodontidae) to which *Spelerpes* belongs, adaptations to a subterranean water life having been added.

The derotremes also are not primitive urodeles. Since they show a mixture of the characters of the larvae and grown-up salamanders, one cannot derive them from typical larvae. Presumably the derotremes are descended from typical salamanders which have returned completely to aquatic life. Thus the metamorphosis, which serves to adapt the larvae to land life, has lost its biological meaning; it extends over a longer time, and finally sexual maturity overtakes the yet imperfectly built animal. Some organs complete the metamorphosis, others retain wholly or in part the larval condition. So in its skull *Cryptobranchus* (Reese '06) resembles the grown-up salamander; in its circulation it retains larval characters. Versluys believes *Cryptobranchus* to be descended from the amblystomidae.

If we take into account only living forms, much of the data thus far considered in seeking a solution of this general question of the relationship of the aquatic and land-living urodeles may be read either way. The key to the situation lies in the comparison of the structures of existing urodeles with those of fossil forms. We have seen that the extinct ancestor of the urodeles was probably an animal whose skull was more complete than that of any living urodele. In *Necturus* (Kingsbury '05), there is a considerable reduction in the number of cranial bones, comparable to the condition in the larval *Spelerpes*, and contrasting with the condition in the adult *Spelerpes*. If the stem-form of the urodeles was an animal like *Branchiosaurus*, then so far as the cranial elements are concerned the salamander, and not the perennibranch, is the more primitive form. There are five branchial arches in *Branchiosaurus*—a condition which does not exclude either view of the interrelationships of the urodeles. *Branchiosaurus* had external gills only in the larval stage; this indicates that the condition found in the perennibranchs is probably not in the ancestral line of the caducibranchs (*salamandrina*).

Von Eggeling ('11), from a comparative study of the histogenesis of the skeleton of the limbs of urodeles, favors the view that the perennibranchs, derotremes and *siredon* are derived from the caducibranchs.

With the transition from the water to the land, or vice versa, one might expect important modifications in the sense organs. In the amphibia this expectation is best realized in the auditory organs, which in the form of a columellar apparatus fitting into a fenestra ovalis here occur for the first time in the vertebrate series. From a study of the modifications of this apparatus we may well hope to obtain information concerning the more intimate phylogenetic relationships within the group. According to the recent work of Kingsbury and Reed ('09) on the urodeles, there is close correlation between the type of auditory apparatus present and the habits (aquatic, semi-aquatic, terrestrial, burrowing, etc.) of the animals. From their results one may note many resemblances between the adults of the typically aquatic forms and the aquatic larvae of the terrestrial forms, as well as contrasts between both of these and the adult terrestrial forms. Concerning the phylogenetic relationships as indicated by the auditory apparatus Reed ('09) says:

Cryptobranchus is the most generalized. The *amblystomidae* are intermediate between *Cryptobranchus* and all other groups. The *plethodontidae* and *desmognathidae* are departures from the *Amblystoma* stem while from these the *sirenidae* and *Amphiuma* seem to be degenerated. *Diemyctylus* and *Triton* are identical with regard to these ear structures and differ from all others. They are to be considered the most specialized. Between *Diemyctylus* and *Triton* on the one hand and the *amblystomidae* on the other *Salamandra* stands intermediate, resembling more strongly the *amblystomidae*.

If the generalized condition is really a primary one, then so far as the evidence from this single character is concerned, *Cryptobranchus* is one of the most primitive of urodeles; the evidence is not in line with the hypothesis of Versluys. But one should seriously consider whether the correlation between the sound-transmitting organs and the environmental relations is not too close to make the character of much phylogenetic value.

Osborn ('88) states that in *Cryptobranchus* we have the most primitive type of brain thus far observed among the amphibia. But it is not clear that the simple condition found is necessarily primitive.

Whipple ('06), from a comparative study of the ypsiloid apparatus in urodeles reaches the following conclusions:

(a) "That forms with lungs but without vestiges of an ypsiloid apparatus, and with no evidence of degeneration in the pelvic region (e.g., *Necturus*) are neither degenerate forms, nor permanent larvae of any of the salamandrina."

(b) "That the presence of a functional ypsiloid apparatus in *Cryptobranchus* indicates that *Cryptobranchus* lies near the line of descent of the salamandrina."

In summing up the facts for and against the hypothesis of the phylogenetic relationships of the perennibranchs, derotremes and salamandrina as outlined by Versluys, it seems to me that the arguments in favor of the hypothesis are founded on characters of greater phylogenetic value. In the reptiles and mammals, land forms are always primitive, aquatic forms secondary (Osborn '02). To the writer the evidence seems convincing in favor of a similar view for the recent urodeles. It might be added that pentadactylous limbs, more or less perfectly developed in fossil as well as recent amphibia, were undoubtedly produced in connection with terrestrial habits; and it should be emphasized that the forms ancestral to the present-day aquatic urodeles were probably not purely terrestrial, but passed through an aquatic larval stage, as in *Branchiosaurus* and most of the living salamanders.

It remains to consider briefly the phylogenetic value of some of the facts concerning the life cycle of *Cryptobranchus* that are embodied in the present contribution, and to discuss their bearing on the subjects just treated from a historical point of view. In an investigation thus far confined mainly to the external features of development, manifestly little more than a beginning can be made in such an interpretation.

The repeated failure of embryological generalizations to solve some of the larger phylogenetic problems led to a widespread reaction against the earlier too sweeping conclusions based on the recapitulation theory. The reproductive processes, while so fundamental, are very plastic, modified in closely related species and even changing somewhat in the same species kept under

different conditions. A strict application of the biogenetic law would imply that the early stages of development are exclusively palingenetic; but from the earliest stages we find adaptations (e.g., the presence of yolk) that are prospective in their significance and have to do with distinctively larval phases, while the larval characters may be highly coenogenetic. It is the failure to distinguish between palingenetic and coenogenetic features of development that is mainly responsible for bringing the recapitulation theory into disrepute. With a clearer recognition of the limitations in this field of study, we may yet question whether the reaction against the validity of the recapitulation theory has not gone too far. As compared with anatomy and paleontology, embryology is doubtless of little service in connecting up the great groups of animals; yet it is indispensable in the solution of many special problems.

In a comparative study of the breeding habits (Part I, Smith '12) we must remember that we are dealing with characters that occur very late in the ontogeny, having to do with the adult rather than with the developing animal. Consequently such characters are of no more phylogenetic value than the habits in general and the family, generic and specific morphological characteristics of the adult; they are exceedingly plastic and of value for comparison only within a very limited range of forms.

A phylogenetic interpretation of the methods of fertilization in urodeles has been given in a previous paper (Smith '07 b); it is here referred to with the remark that in view of the conclusions reached in the present paper regarding the trend of evolution in the urodeles, the series should be reversed. If we could go back far enough in the phylogeny of the vertebrates, doubtless we should find external fertilization to be the primitive condition (e.g., as in *Amphioxus*); but it is entirely possible that in *Cryptobranchus* the method is secondarily acquired.

As we should expect, the brooding habit of *Cryptobranchus* is very similar to that of the closely-related *Amphiuma*. The absence of a brooding habit in *Necturus* is noteworthy. Brooding habits very similar to that of *Cryptobranchus* are found in *Desmognathus* and *Plethodon*, but with this important differ-

ence: in these latter forms the female, not the male, cares for the eggs.

The egg capsules of the urodeles, as in other groups, show generic and even specific differences (e.g., the specific differences in the egg masses of *Amblystoma*, Smith '11 b). This is what might be expected, since the capsules are the product of the soma, not of the germ cells; the facts are in no way incompatible with von Baer's law. The close resemblance between the egg envelopes of *Cryptobranchus* and *Amphiuma* accords with the systematic relationship. Amongst terrestrial urodeles there is no close approach to the type found in *Cryptobranchus*; but in general we should seek for affinities in forms having the egg capsules more or less independent in the cluster, connected by stalks (e.g., as in *Desmognathus*, Wilder '99), rather than in forms in which the individual capsules are surrounded by a common jelly mass, as in *Amblystoma*.

The origin of the follicle cells of *Cryptobranchus* has been traced (Part I) from the epithelial cells of the ovarian wall. Hence these cells belong to the soma, and the rather marked differences between the follicle cells of *Cryptobranchus* and *Necturus* are of value for comparison only with a very limited range of forms.

Taking up the history of the egg proper, we first note that the progressive change of the ovarian egg from anolecithal through an isolecithal to a telolecithal stage is a recapitulation of a very ancient series of events in the phylogeny. The actual amount of yolk present is a coenogenetic character, for the yolk content changes greatly within nearly related forms.

The factors that determine the amount of yolk present in the eggs of different species are complex and do not readily fall under any single law. Protection of the eggs through nesting and brooding habits makes possible a reduction in their number, enabling the female to endow each egg with a larger store of yolk, thereby giving the young a better start in life. Such a store of yolk allows development to go further before the young animal is cast upon its own resources, so that the necessity for peculiarly larval adaptations is minimized. In purely terrestrial

non-placental forms a large amount of yolk is certainly necessary, for the food (e.g., insect larvae) of the young is larger and less easy to capture than is the case with aquatic larvae. On the other hand a large store of yolk is common in the eggs of fishes. The most we can infer is that the presence of a large amount of yolk in the egg is one of the conditions that makes possible the invasion of the land; it cannot be said that the presence of an unusual amount of yolk in the egg of *Cryptobranchus* is evidence either for or against a terrestrial ancestry.

Among the known eggs of urodeles that of *Cryptobranchus* is probably the most heavily yolk-laden; the egg of *Necturus* contains nearly as much yolk. In the salamandrina one notes the large amount of yolk in the eggs of *Desmognathus* (Wilder '04; Hilton '04 and '09); *Spelerpes* (Goodale '11) and *Plethodon* (Piersol '09).

The absence of pigment in the egg of *Cryptobranchus* is correlated with the nesting habits, whereby the eggs are protected from the light. Similar conditions are found in *Necturus*, *Desmognathus*, *Plethodon* and *Spelerpes*.

The occurrence of a protoplasmic mantle and cytodisc in the late ovarian egg of *Cryptobranchus* parallels a condition in the teleost egg which reaches its full expression just before fertilization; in *Cryptobranchus* this condition is transient. The segregation of a definite layer of cytoplasm close to the surface of the blastodisc in *Cryptobranchus* shortly after fertilization suggests a parallel with the marked increase in thickness of the germinal disc of the teleost egg immediately after fertilization; Professor Dean informs me that he has observed a similar phenomenon in ganoids.

The fundamental features of the early embryonic development have to do with the building up of the very general structures and body relations common to all vertebrates. In their most general aspects cleavage and gastrulation are extremely palinogenetic phases of development. But we find secondarily imposed on the essential features of these processes, modifications which are highly coenogenetic and of adaptive significance mainly for the embryo and larva (Lillie '98), rather than for the adult.

These stages seem to possess few characters intermediate between the extremes indicated, consequently they tell us little about the relationships of the larger groups; but the coenogenetic characters, which are plastic and vary widely within a limited range, may be of value for determining relationships within these groups. The most striking of these coenogenetic characters are correlated with the presence of yolk (Conklin '07).

In the highly telolecithal and heavily yolk-laden egg of *Cryptobranchus*, we may interpret holoblastic cleavage as a persistent primitive character. For were the ancestral form one with meroblastic cleavage, we should hardly expect the holoblastic method to arise under such unfavorable conditions.

Comparisons between the early cleavage furrows of different urodeles seem justified on the ground that we are comparing cells of the same generation; but in view of the highly indeterminate character of the cleavage (Jordan and Eycleshymer '94) such comparisons do not take us very far. Mechanical factors doubtless play a part (McMurrich '94), but these mechanical factors are conditioned by the organization of the egg, which is hereditary.

There is close correlation between the method of third cleavage and the yolk content. A vertical third cleavage is characteristic of heavily yolk-laden and highly telolecithal eggs; a latitudinal third cleavage is found rather in eggs with yolk both smaller in amount and with a lesser degree of segregation from the cytoplasm. Morgan ('93) has shown that in teleost eggs from which yolk has been experimentally removed, the third cleavage often comes in latitudinally, yet the eggs produce perfect embryos. Marked variation in the direction of the third cleavage furrows occurs in eggs in which the conditions are intermediate in character; the egg is oscillating between two possible modes of cleavage. A vertical third cleavage is characteristic of the egg of *Cryptobranchus*; it is found less uniformly in the eggs of *Desmognathus*; in *Necturus* and *Diemyctylus* the third cleavage is irregular; in *Amblystoma* it is latitudinal. So far as this character is concerned *Cryptobranchus* lies nearest to *Desmognathus* and is most remote from *Amblystoma*.

The important features of the later stages of the cleavage have to do with processes that are not well expressed in the superficial cleavage pattern: migration of cells and the various processes of differentiation that lead up to gastrulation and early embryo-formation.

The occurrence of a septal furrow in the gastrula stages of two such widely-separated forms as *Cryptobranchus* and *Petromyzon* is a remarkable case of convergence in purely embryonic characters. The septal furrow and fenestra are the mechanical product of gastrulation by invagination and epiboly in a heavily yolk-laden egg with a very thin roof to the blastocoele. So far as known these features of gastrulation in *Cryptobranchus* are unique among urodeles; but there is evidently an approach to this condition in *Spelerpes*, since the egg is heavily yolk-laden and during gastrulation the blastocoele roof becomes quite thin (Goodale '11).

The study of the later embryonic and larval stages is as yet too superficial to furnish much data for phylogenetic generalization; yet for this purpose the late stages will probably prove of greater value than the earlier ones (Wilson '98, p. 23). It should be particularly noted that the larval *Cryptobranchus* reaches an age of two years before transforming—evidence of a retarded metamorphosis. Reasons have already been given for believing that the metamorphosis is incomplete.

The study of the breeding habits, the organization of the eggs, and the early course of development lead us to look among the land-living salamanders for affinities to *Cryptobranchus*—more particularly to forms like *Desmognathus*, *Spelerpes* and *Plethodon*. Considerable evidence from comparative anatomy, particularly with regard to skull structure, will be found to harmonize with this view. *Desmognathus* in particular is suggestive; according to Kingsbury ('02) it is semi-aquatic in its habits, living at the edges of swiftly running brooks. It conceals itself under stones at the edge of the stream or in its immediate vicinity, and here its unpigmented and heavily yolk-laden eggs are laid. There is a brooding habit, though in this case the female guards the eggs; we have noted some marked similarities to *Cryptobranchus* in the early development. Yet we are hardly warranted in con-

cluding that the relationship is very close. In particular we should expect the immediate ancestral stock of *Cryptobranchus* and the closely related miocene fossil *Andrias scheuchzeri* (Gadow '01, p. 84) to consist of larger animals.

According to the view adopted in this paper, the urodeles, very remotely descended from aquatic stock, are primarily terrestrial, but with aquatic larvae. On the land they were unsuccessful in the struggle for existence in the open; they took refuge in sheltered situations, and for this they have paid the penalty of degeneration. Yet, in the main, the result of the arrest of a typical terrestrial adaptive radiation has been the retention of primitive characters. Some became secondarily aquatic; this is one phase of the tendency toward secluded habits, and involves the retention of larval characters. Added to these more conspicuous peculiarities we find a great variety of special adaptations to a retired mode of life, some of them correlated with the defenseless condition of the animals. Of all these changes, reversion to an aquatic mode of life is a factor which, cutting across many lines of descent, has done most to disguise the real relationships, and of this we have a conspicuous example in *Cryptobranchus*.

BIBLIOGRAPHY

A few titles are included that are not directly referred to in the text.

- AGASSIZ, L., AND WHITMAN, C. O. 1884 On the development of some pelagic fish eggs. *Proc. Amer. Acad. Arts and Sciences*, vol. 20.
- ASSHETON, RICHARD 1896 Notes on the ciliation of the ectoderm of the amphibian embryo. *Quart. Jour. Micros. Sci.*, vol. 38.
- BAUR, G. 1896 The stegocephali. A phylogenetic study. *Anat. Anz.*, Bd. 11.
- BLOUNT, MARY 1907 The early development of the pigeon's egg, with especial reference to the supernumerary sperm nuclei, the periblast and the germ wall. *Biol. Bull.*, vol. 13.
- BOAS, J. E. V. 1881 Ueber den Conus Arteriosus und die Arterienbogen der Amphibien. *Morph. Jahrb.*, Bd. 7.
- 1896 Ueber Neotenie. *Festschr. f. Carl Gegenbaur*, Bd. 2.
- BRAUER, AUGUST 1899 Beiträge zur Kenntniss der Entwicklung und Anatomie der Gymnophionen. II. Die Entwicklung der aussern Form. *Zool. Jahrb.*, Abth. Anat., Bd. 12.
- BUDGETT, JOHN SAMUEL 1901 On the breeding habits of some West African fishes, with an account of the external features in the development of *Protopterus annectens*, and a description of the larvae of *Polypterus lapradei*. *Trans. Zool. Soc. London*, vol. 16, part 2, August. Also Budgett memorial volume, part VI (Kerr '07).
- CONKLIN, E. G. 1907 The embryology of *Fulgur*: A study of the influence of yolk on development. *Proc. Phila. Acad. Sci.*, July.
- COPE, E. D. 1885 The retrograde metamorphosis of *Siren*. *Amer. Nat.*, vol. 19.
- 1889 The batrachia of North America. *Bull. U. S. Nat. Mus.*, no. 34.
- CREDNER, HERMANN 1881-1890 Die Stegocephalen und Saurier aus dem rothliegenden des Plauen'schen Grundes bei Dresden. R. Friedlander und Sohn, Berlin.
- DEAN, BASHFORD 1895 Early development of gar-pike and sturgeon. *Jour. Morph.*, vol. 11.
- 1896 The early development of *Amia*. *Quart. Jour. Micros. Sci.*, vol. 38.
- DE LANGE, DAN., JR. 1907 Die Keimblätterbildung des *Megalobatrachus maximus* Schlegel. *Anat. Hefte*, Bd. 32.
- DE BUSSY, L. P. 1904 Eerste Ontwikkelingsstadiën van *Megalobatrachus maximus* Schlegel. *Tijdschrift der nederlandsche Dierkundige Vereeniging*, Bd. 8.
- 1905 Die ersten Entwicklungsstadien des *Megalobatrachus maximus*. *Zool. Anz.*, Bd. 28.
- EGGELING, H. VON 1911 Der Aufbau der Skeletteile in den freien Gliedmassen der Wirbeltiere. *Untersuchungen an urodelen Amphibia*. Gustav Fischer, Jena. (Reviewed by Bardeen in *Science*, September 29).
- EMERSON, E. T. 1905 General anatomy of *Typhlomolge rathbuni*. *Proc. Boston Soc. Nat. Hist.*, vol. 32.
- EYCLESYMER, ALBERT C. 1895 The early development of *Amblystoma*, with observations on some other vertebrates. *Jour. Morph.*, vol. 10.
- 1898 The location of the basis of the amphibian embryo. *Jour. Morph.*, vol. 14.

- EYCLESHYMER, ALBERT C. 1899 The cleavage of the egg of *Lepidosteus osseus*. *Anat. Anz.*, Bd. 16.
 1902 The formation of the embryo of *Necturus*, with remarks on the theory of concrescence. *Anat. Anz.*, Bd. 21.
 1904 Bilateral symmetry in the egg of *Necturus*. *Anat. Anz.*, Bd. 25.
- EYCLESHYMER, A. C., and WILSON, JAMES M. 1910 Normal plates of the development of *Necturus maculosus*. *Keibel, Normentafeln zur Entw. der Wirbeltiere*, Heft 11. Gustav Fischer, Jena.
- GADOW, HANS 1901 *Amphibia and reptiles*. Cambridge Nat. Hist., vol. 8.
- GOETTE, ALEXANDER 1875 *Die Entwicklungsgeschichte der Unke*. Mit einem Atlas. Leopold Voss, Leipzig.
- GÖLDI, EMIL A. 1899 Über die Entwicklung von *Siphonops annulatus*. *Zool. Jahrb., Abth. Syst.*, Bd. 12.
- GOODALE, H. D. 1911 The early development of *Spelerpes bilineatus* Green. *Amer. Jour. Anat.*, vol. 12.
- GOODRICH, E. S. 1909 *Vertebrata craniata*. Part IX of Lankester's treatise on zoology. Adam and Charles Black, London.
- GREGORY, W. K. 1907 The orders of teleostomous fishes. A preliminary review of the broader features of their evolution and taxonomy. *Annals N. Y. Acad. Sci.*, vol. 17, Part II, no. 3.
 1911a The limbs of *Eryops* and the origin of paired limbs from fins. Secretary's report of the meeting of the New York Academy of Sciences, Section of Biology, Feb. 13. *Science*, vol. 33, Mar. 31.
 1911b Further notes on the evolution of paired fins. Secretary's Report of the meeting of the New York Academy of Sciences, Section of Biology, Oct. 16. *Science*, vol. 34, no. 886, Dec. 22.
 1912 The origin of paired limbs. Secretary's report of the meeting of the New York Academy of Sciences, Section of Biology, Dec. 11, 1911. *Science*, vol. 30, Apr. 12.
- GRIGGS, LELAND 1910 Early stages in the development of the central nervous system of *Amblystoma punctatum*. *Jour. Morph.*, vol. 21, no. 3.
- HATTA, S. 1907 *Gastrulation in Petromyzon*. *Jour. Coll. Sci.*, Tokyo.
- HERTWIG, OSCAR 1906 *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere*. Gustav Fischer, Jena.
- HILL, CHARLES 1899 Primary segments of the vertebrate head. *Anat. Anz.*, Bd. 16.
 1900 Developmental history of the primary segments of the vertebrate head. *Zool. Jahrb., Abth. für Anatomie und Ontog.*, Bd. 13.
- HILTON, WM. A. 1904 Segmentation of the ovum of *Desmognathus fusca*. *Amer. Nat.*, vol. 38.
 1909 General features of the early development of *Desmognathus fusca*. *Jour. Morph.*, vol. 20.
- IKEDO, S. 1902 Contributions to the embryology of the amphibia: The mode of blastopore closure and the position of the embryonic body. *Jour. Coll. Sci.*, Tokyo, vol. 17, Part II.
- ISHIKAWA, C. 1904 Beiträge zur Kenntniss des Riesen-Salamanders (*Megalobatrachus maximus* Schlegel). *Proc. Dept. Nat. Hist.*, Tokyo Imperial Museum, vol. 1, no. 2.

- ISHIKAWA, C. 1908 Ueber den Riesen-Salamander Japans. Mitteilungen der deutschen Gesellschaft für Natur-und Völker-kunde Ostasiens. Bd. 11, Th. 2.
1909 Note on the gastrulation of the giant salamander, *Megalobatrachus sieboldii*. Proceedings of the seventh international zoological congress, Boston, Aug. 19-24, 1907.
- JORDAN, EDWIN O. 1893 The habits and development of the newt. Jour. Morph., vol. 7.
- JORDAN, E. O. and EYCLES HYMER, A. C. 1894 On the cleavage of the amphibian ovum. Jour. Morph., vol. 9.
- KELLICOTT, W. E. 1905a The development of the vascular and respiratory systems in *Ceratodus*. N. Y. Acad. Sci. Memoirs, vol. 2, Part IV.
1905b The development of the vascular system of *Ceratodus*. Anat. Anz., vol. 26.
- KERR, J. GRAHAM 1900 External features in the development of *Lepidosiren*. Philos. Trans. Royal Soc., vol. 192, B.
1901 The development of *Lepidosiren paradoxa*. II. With a note upon the corresponding stages in the development of *Protopterus annectens*. Quart. Jour. Micros. Sci., vol. 45.
1907a The development of *Polypterus senegalus* Cuv. Budgett memorial volume (Kerr, '07b).
1907b The work of John Samuel Budgett. Cambridge Univ. Press.
1909 Normal plates of the development of *Lepidosiren paradoxa* and *Protopterus annectens*. Keibel, Normentafeln zur Entw. der Wirbeltiere, Heft 10. Gustav Fischer, Jena.
- KINGSBURY, B. F. 1902 The spermatogenesis of *Desmognathus fusca*. Amer. Jour. Anat., vol. 1.
1905 The rank of *Necturus* among tailed batrachia. Biol. Bull., vol. 8.
- KINGSBURY, B. F. and REED, H. D. 1909 The columella auris in amphibia. Jour. Morph., vol. 20.
- KINGSLEY, J. S. 1899 Text-book of vertebrate zoology. Henry Holt and Co., New York.
1901 Amphibian studies. Abstract of paper read before the American Morphological Society. Biol. Bull., vol. 2.
- KUNITOMO, KANAE 1910 Ueber die Entwicklungsgeschichte des *Hynobius nebulosus*. Anat. Hefte, Beitr. und Ref. Anat. und Entw., Bd. 40.
- KUPFFER, C. 1885 Primäre Metamerie des Neuralrohrs der Vertebraten. Sitzungsber. der mathem.-physik. Kl. der K. B. Akad. d. W. München.
1892 Entwicklungsgeschichte des Kopfes. Merkel und Bonnet, Ergebnisse der Anatomie, Bd. 2.
1893 Studien zur vergleichenden Entwicklungsgeschichte des Kopfes der Kranioten. Heft 1. München und Leipzig.
1903-1905 Die Morphologie des Centralnervensystem. Handbuch der vergl. und exp. Entwicklungslehre der Wirbeltiere, Hertwig, '06.
- LILLIE, FRANK R. 1898 Adaptation in cleavage. Woods Hole biological lectures, 1897-1898.
1908 Development of the chick. Henry Holt and Co.

- Locy, W. A. 1895 A contribution to the structure and development of the vertebrate head. Jour. Morph., vol. 11.
- McCLENDON, J. F. 1910 The development of isolated blastomeres of the frog's egg. Amer. Jour. Anat., vol. 10.
- McGREGOR, J. H. 1897 An embryo of *Cryptobranchus*. Anat. Anz., Bd. 13.
- McMURRICH, J. P. 1894 Cell division and development. Woods Hole biological lectures.
- MOODIE, ROY L. 1908a The lateral line system of extinct amphibia. Jour. Morph., vol. 19.
- 1908b The ancestry of the caudate amphibia. Amer. Nat., vol. 42.
- MORGAN, T. H. 1893 Experiments on teleost eggs. Anat. Anz., Bd. 8.
- 1904 Die Entwicklung des Froscheies. Wilhelm Engelmann, Leipzig.
- ORB, H. 1887 A contribution to the embryology of the lizard. Jour. Morph., vol. 1.
- OSBORN, H. F. 1888 A contribution to the internal structure of the amphibian brain. Jour. Morph., vol. 2.
- 1902 The law of adaptive radiation. Amer. Nat., vol. 36.
- PARKER, W. K. 1877 On the structure and development of the skull in the urodelous amphibia. Part I. Philos. Trans. Royal Soc., vol. 167, Part II.
- PARKER, T. JEFFERY, and HASWELL, WM. A. 1897 A text-book of zoology, vol. 2. The Macmillan Co.
- PIERSOL, W. H. 1909 The habits and larval state of *Plethodon cinereus erythronotus*. Trans. Canadian Institute, vol. 8, Part IV.
- REED, H. D. 1909 Systematic relations of the urodela as interpreted by a study of the sound-producing organs. Science, Apr. 30.
- REESE, ALBERT M. 1906 Anatomy of *Cryptobranchus allegheniensis*. Amer. Nat., vol. 40.
- ROBINSON, ARTHUR, and ASSHETON, RICHARD 1891 The formation and fate of the primitive streak, with observations on the archenteron and germinal layers of *Rana temporaria*. Quart. Jour. Micros. Sci., N. S., vol. 32.
- SCHULTZE, O. 1895 Die künstliche Erzeugung von Doppelbildung bei Frosch-Larven mit Hilfe abnormer Gravitationswirkung. Archiv Entw., Bd. 1.
- 1900 Ueber das erste Auftreten der bilateralen Symmetrie im Verlauf der Entwicklung. Archiv für mikr. Anat., Bd. 55.
- SEMON, R. 1900 Die Furchung und Entwicklung der Keimblätter bei *Ceratodus forsteri*. Aus Semon, zoologische Forschungsreisen in Australia und dem Malayischen Archipel, Bd. 4. Gustav Fischer, Jena.
- 1901 Normentafeln zur Entw. des *Ceratodus forsteri*. Keibel, Normentafeln zur Entw. der Wirbeltiere, Heft 3. Gustav Fischer, Jena.
- SMITH, BERTRAM G. 1906 Preliminary report on the embryology of *Cryptobranchus allegheniensis*. Biol. Bull., vol. 11.
- 1907 a The life history and habits of *Cryptobranchus allegheniensis*. Biol. Bull., vol. 13.
- 1907 b The breeding habits of *Amblystoma punctatum* Linn. Amer. Nat., vol. 41.
- 1911 a The nests and larvae of *Necturus*. Biol. Bull., vol. 20.

- SMITH, BERTRAM G. 1911 b Notes on the natural history of *Amblystoma jeffersonianum*, *A. punctatum* and *A. tigrinum*. Bull. Wis. Nat. Hist. Soc., vol. 9.
- 1912 The embryology of *Cryptobranchus alleganiensis*, including comparisons with some other vertebrates. Part 1: Introduction; the history of the egg before cleavage. Jour. Morph., vol. 23.
- SPEMANN, H. 1901-1903 Entwicklungsphysiologische Studien am Triton-Ei. I, II, III. Archiv Entw., 12, 15, 16.
- STANNIUS, H. 1856 Handbuch der Zootomie. Berlin.
- STOCKARD, CHARLES, R. 1906 The development of *Fundulus heteroclitus* in solutions of lithium chlorid, with appendix on its development in fresh water. Jour. Exp. Zool., vol. 3.
- VERSLUYS, J. 1909 Die Salamander und die ursprünglichsten vierbeinigen Landwirbeltiere. Naturwissenschaftlichen Wochenschrift, Neue Folge, Bd. 8, Nr. 3.
- WATASE, S. 1891 Studies on cephalopods. Jour. Morph., vol. 4.
- WHIPPLE, INEZ L. 1906 The ypsiloid apparatus of urodeles. Biol. Bull., vol. 10.
- WHITMAN, C. O. and EYCLES HYMER, A. C. 1897 The egg of *Amia* and its cleavage. Jour. Morph., vol. 12.
- WIEDERSHEIM, R. 1877 Das Kopfskelett der urodelen. Morph. Jahrb., Bd. 3.
- WILDER, HARRIS HAWTHORNE 1899 *Desmognathus fusca* and *Spelerpes bilineatus*. Amer. Nat., vol. 33.
- 1904 The early development of *Desmognathus fusca*. Amer. Nat., vol. 38.
- WILSON, CHAS. B. 1896 The wrinkling of the frog's eggs during segmentation. Amer. Nat., vol. 30.
- WILSON, E. B. 1898 Cell lineage and ancestral reminiscence. Woods Hole biological lectures.
- WILSON, H. V. 1891 The embryology of the sea bass (*Serranus*). Bull. U. S. Fish Comm., vol. 9.
- ZITTEL, KARL A. VON 1911 Grundzüge der Paläontologie. R. Oldenbourg München und Berlin.

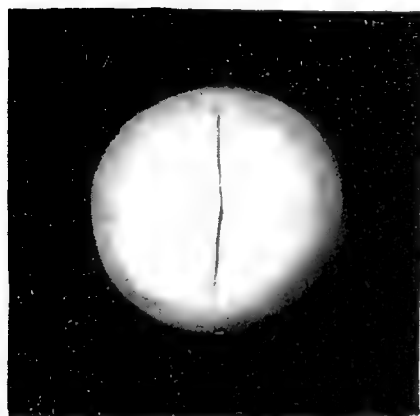
PLATES

PLATE 3

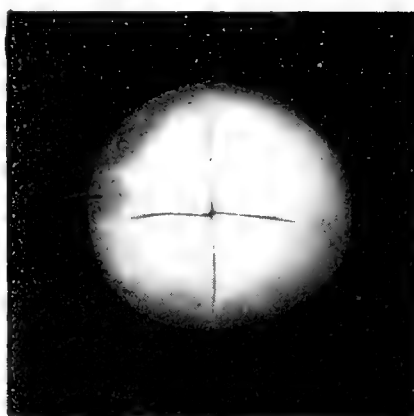
EXPLANATION OF FIGURES (*Cryptobranchus alleggheniensis*)

All the figures are from preserved material, fixed in bichromate-acetic-formalin excepting the eggs shown in figures 210 and 212 which were fixed in formalin. The animal hemisphere is shown in every case. $\times 4$.

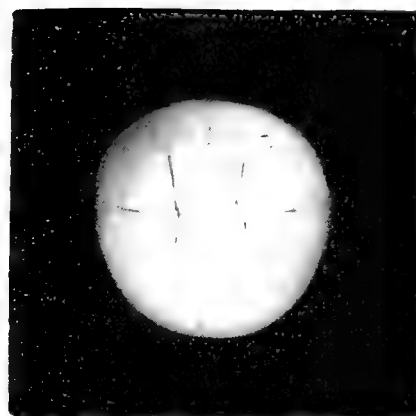
- 204 Stage 1. First cleavage.
- 205 Stage 2. Second cleavage.
- 206 Stage 3. Third cleavage.
- 207 Stage 4. Fourth cleavage.
- 208 Stage 4. Fourth cleavage. Figure 82 is drawn from the same egg.
- 209 Stage 4. Fourth cleavage. Figure 81 is drawn from the same egg.
- 210 Stage 5. Thirteen micromeres. Figure 87 is drawn from the same egg.
- 211 Stage 5. Seventeen micromeres. Figure 86 is drawn from the same egg.
- 212 Stage 6. About thirty-two micromeres. Figure 98 is drawn from the same egg.
- 213 Stage 6. About thirty-two micromeres.
- 214 Stage 6. About thirty-six micromeres.
- 215 Stage 7. About sixty-four micromeres.



204



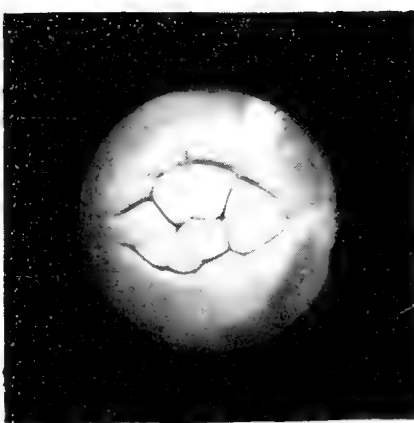
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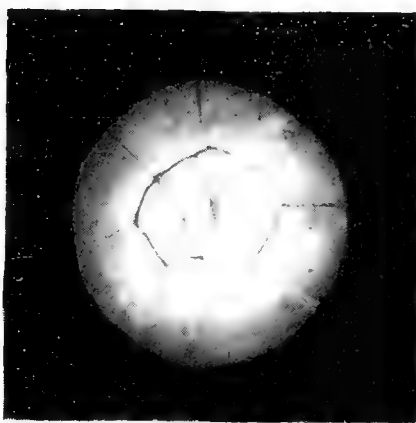
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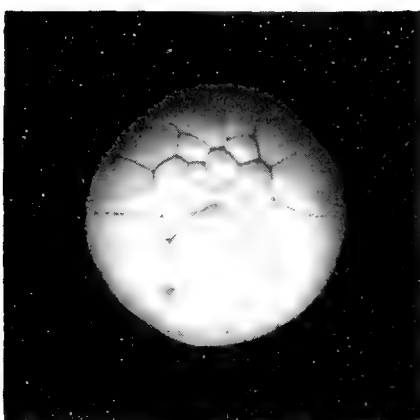
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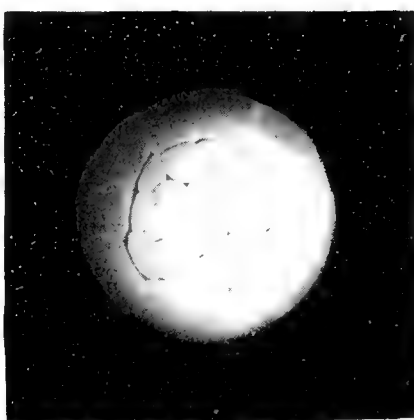
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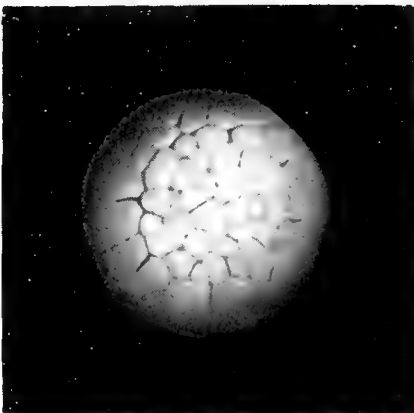
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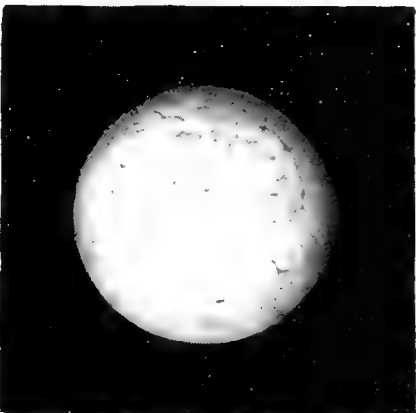
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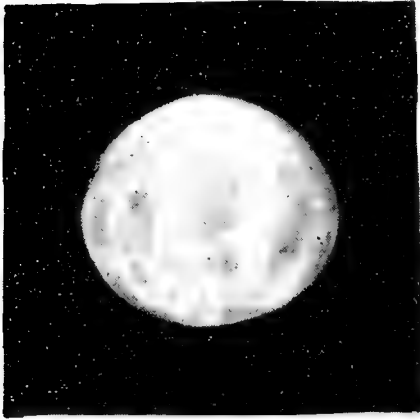
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PLATE 4

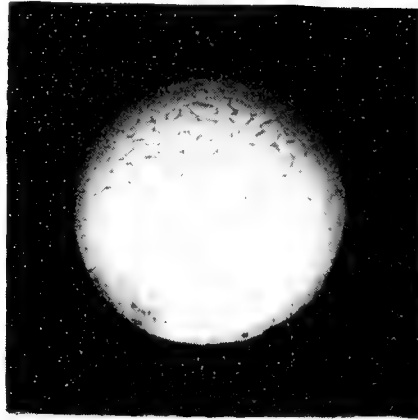
EXPLANATION OF FIGURES (*Cryptobranchus allegheniensis*)

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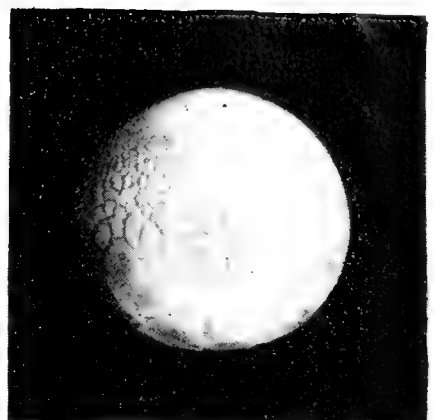
- 216 Stage 7.5. Equatorial view.
- 217 Stage 8.0. Upper hemisphere.
- 218 Stage 9.5. Equatorial view.
- 219 Stage 10.5. Equatorial view of an embryo nearly ready for gastrulation.
- 220 Stage 11.4. Early gastrula, showing the dorsal lip of the blastopore.
- 221 Stage 11.5. Antero-dorsal view of a gastrula, showing the fenestra.
- 222 Stage 11.6. Anterior view of a gastrula, showing the fenestra.
- 223 Stage 12.4. Dorsal view showing early neural plate and neural groove.
- 224 Stage 12.4. Posterior view of the same embryo as in the preceding figure, showing the yolk plug and a part of the neural plate and neural groove.
- 225 Stage 12.5. Dorsal view showing the neural groove and the dorsal lip of the blastopore. Figure 143 is drawn from the same embryo.
- 226 Stage 13.4. Dorsal view showing early neural folds.
- 227 Stage 13.5. Posterior view showing the form of the late blastopore, and the posterior part of the neural plate.



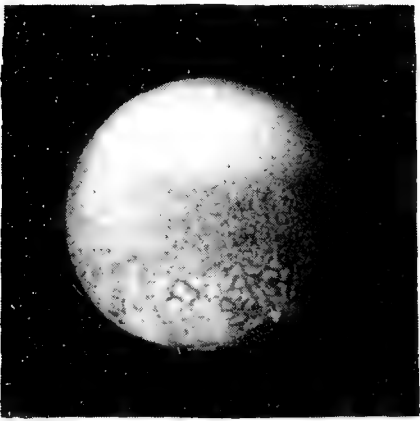
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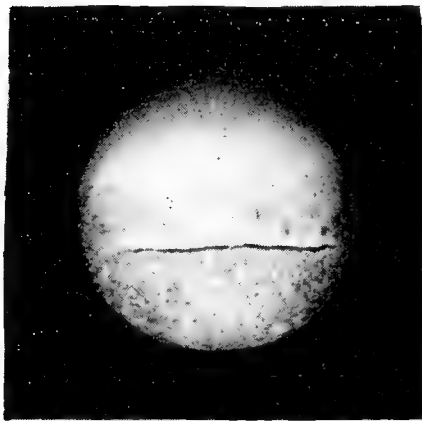
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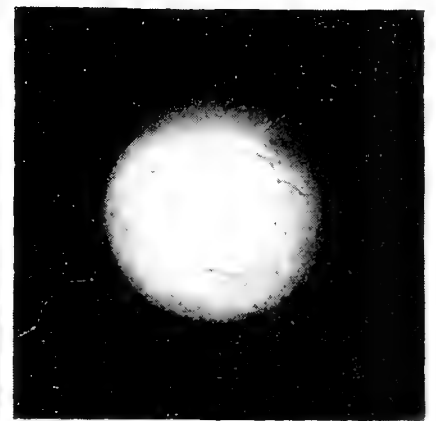
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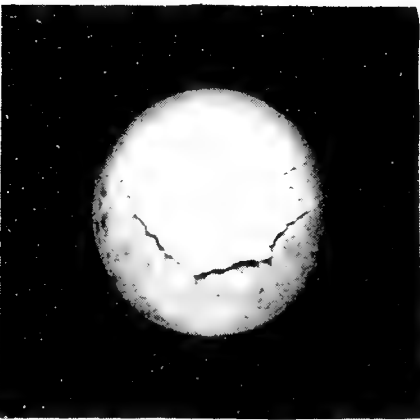
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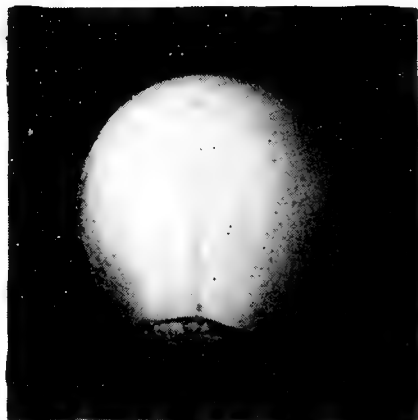
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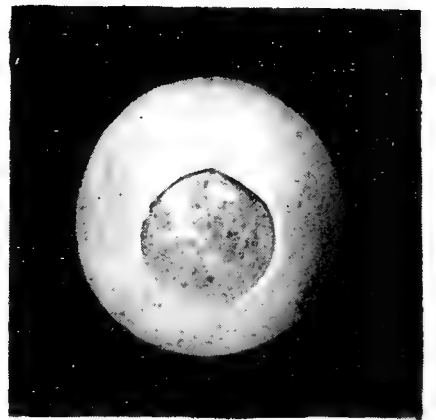
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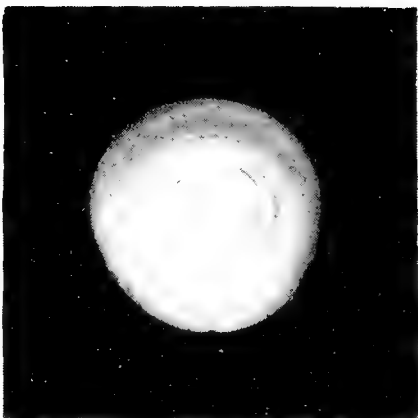
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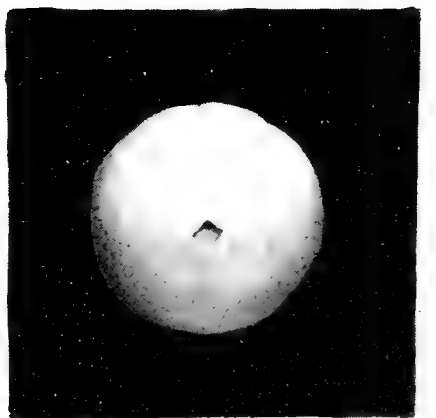
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PLATE 5

EXPLANATION OF FIGURES (*Cryptobranchus allegheniensis*)

Bichromate-acetic-formalin fixation. $\times 4$.

228 Stage 13.5. Posterior view showing the form of the late blastopore, and the posterior part of the neural plate.

229 Stage 14.1. Dorsal view showing neural folds and the early segmentation of the neural plate. Figure 166 is drawn from the same embryo.

230 Stage 14.1. Postero-dorsal view of the same embryo as in the preceding figure, showing the late blastopore and the segmentation of the neural plate.

231 Stage 14.9. Posterior view, showing the late blastopore. Figure 176 is drawn from the same egg.

232 Stage 14.9. Dorsal view of the same embryo as in the preceding figure, showing the segmentation of the neural plate.

233 Stage 15.4. Dorsal view. Figure 184 is drawn from the same egg.

234 Stage 15.8. Dorsal view. Figure 182 is drawn from the same egg.

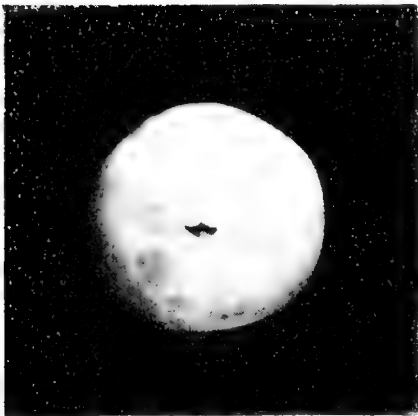
235 Stage 16.0. Dorsal view showing closing neural folds.

236 Stage 16.8. Dorsal view.

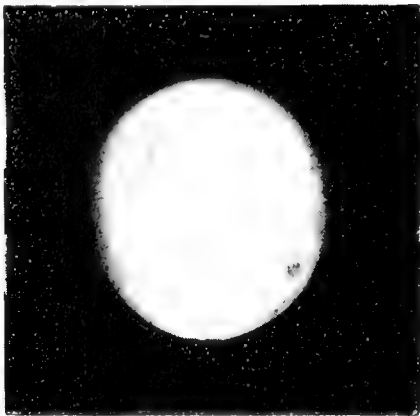
237 Stage 16.8. Antero-dorso-lateral view of the same embryo as in the preceding figure.

238 Stage 17. Antero-dorsal view (inverted with respect to the natural position).

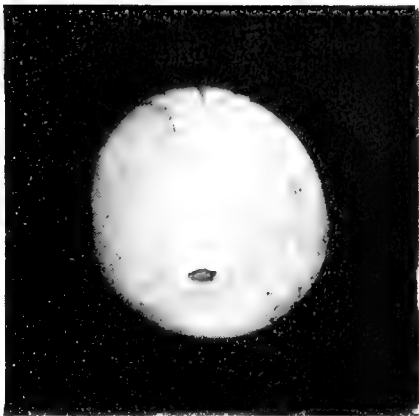
239 Stage 17. Lateral view.



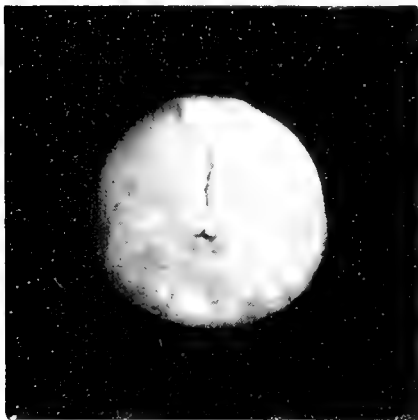
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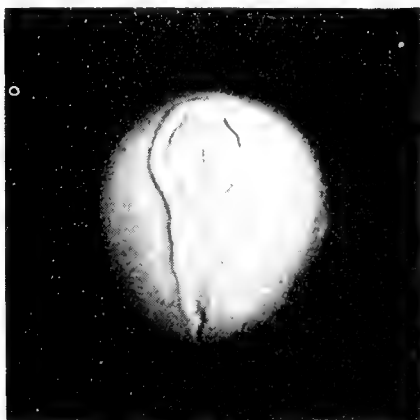
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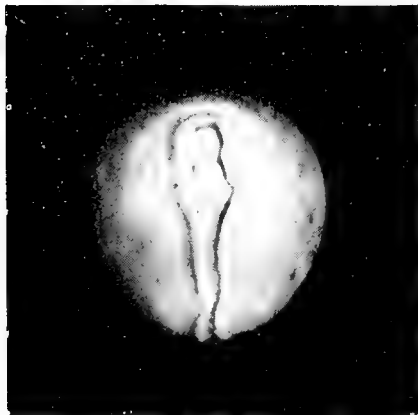
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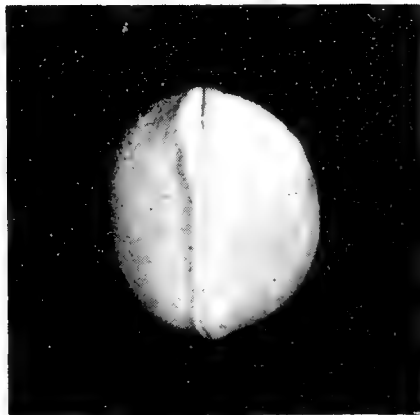
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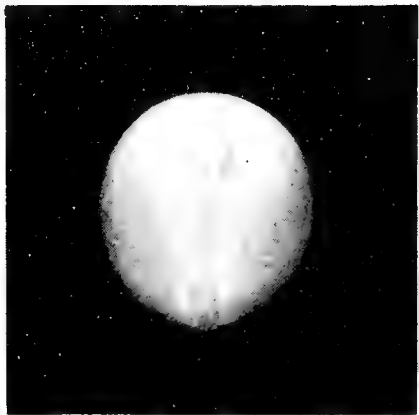
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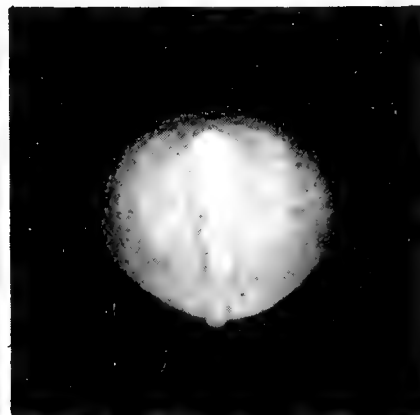
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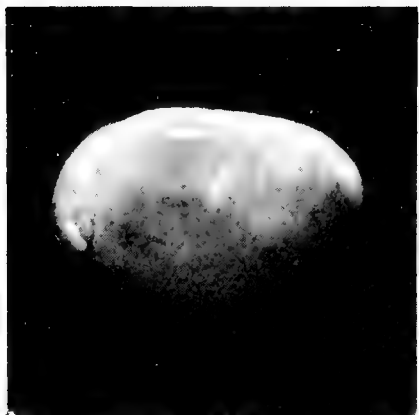
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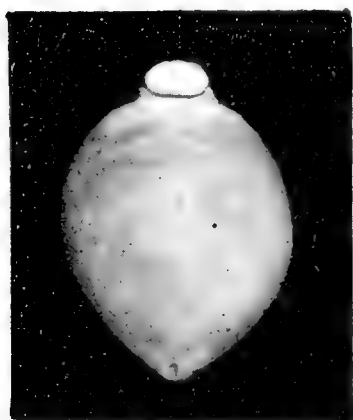
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PLATE 6

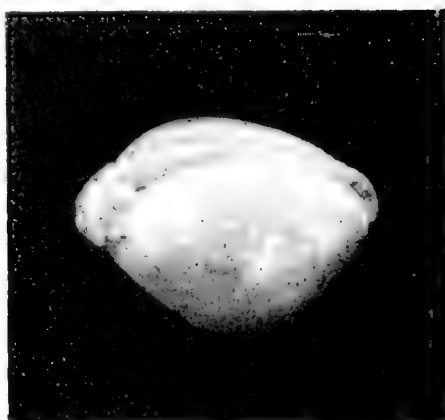
EXPLANATION OF FIGURES (*Cryptobranchus allegheniensis*)

Bichromate-acetic-formalin fixation. $\times 4$.

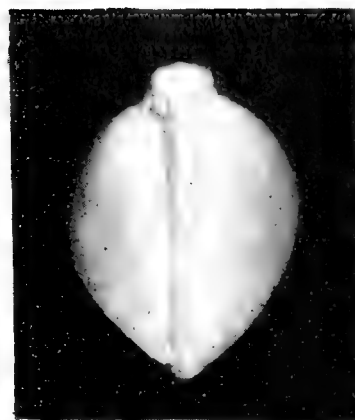
- 240 Stage 17. Ventral view of the embryo shown in the preceding figure.
- 241 Stage 17. Dorso-lateral view of the embryo shown in the preceding figure.
- 242 Stage 17. Dorsal view of the embryo shown in the preceding figure.
- 243 Stage 18. Ventral view.
- 244 Stage 18. Lateral view of the embryo shown in the preceding figure.
- 245 Stage 19. Lateral view.
- 246 Stage 19. Dorsal view.
- 247 Stage 19. Lateral view of the embryo shown in the preceding figure.
- 248 Stage 19. Lateral view.
- 249 Stage 20. Lateral view.



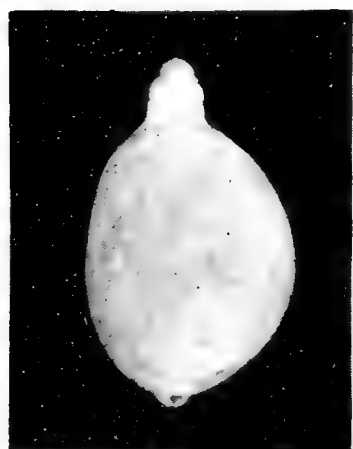
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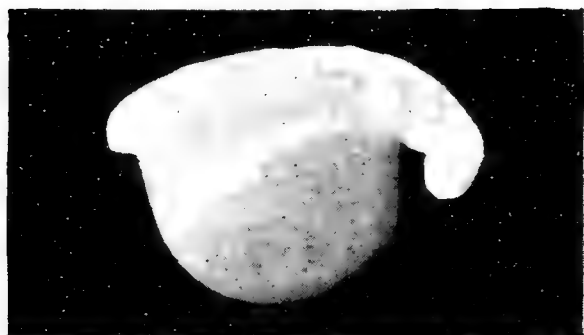
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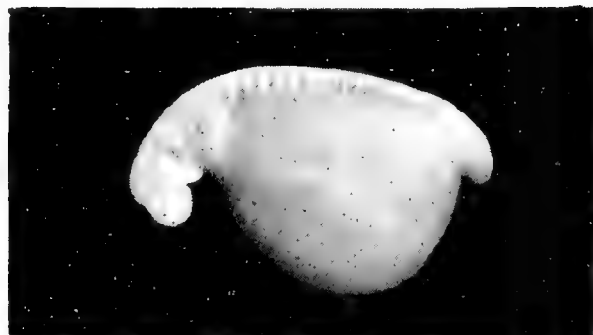
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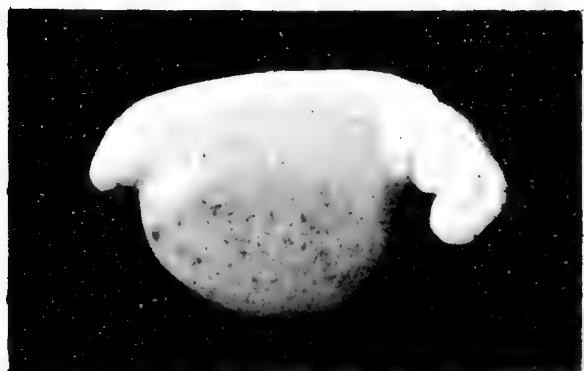
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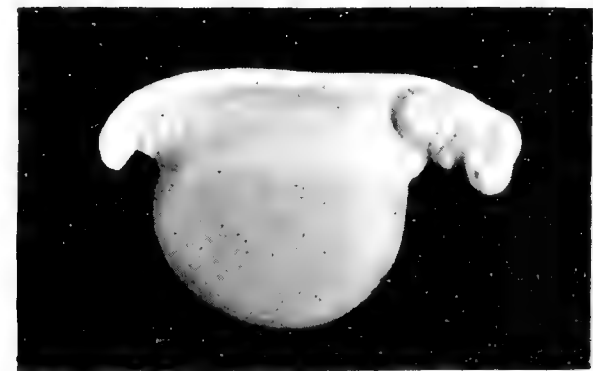
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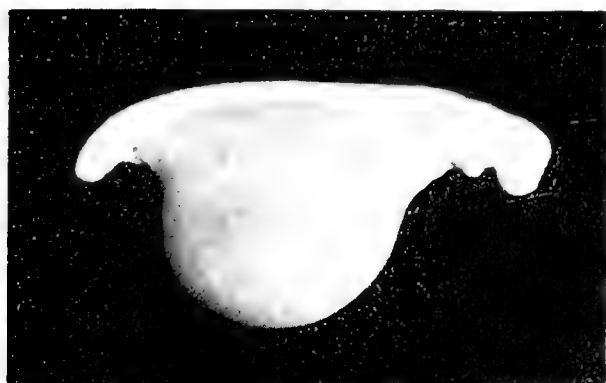
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PLATE 7

EXPLANATION OF FIGURES (*Cryptobranchus allegheniensis*)

Bichromate-acetic-formalin fixation. $\times 4$.

- 250 Stage 20. Lateral view.
- 251 Stage 20. Ventral view of the embryo shown in the preceding figure.
- 252 Stage 21. Ventral view.
- 253 Stage 21. Lateral view.
- 254 Stage 21. Dorsal view of the embryo shown in the preceding figure.
- 255 Stage 21. Ventral view of the embryo shown in the preceding figure.
- 256 Stage 22. Dorsal view.
- 257 Stage 22. Ventral view of the embryo shown in the preceding figure.



250



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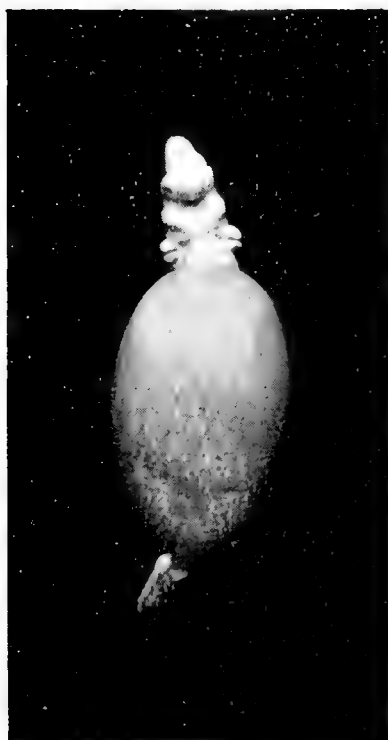
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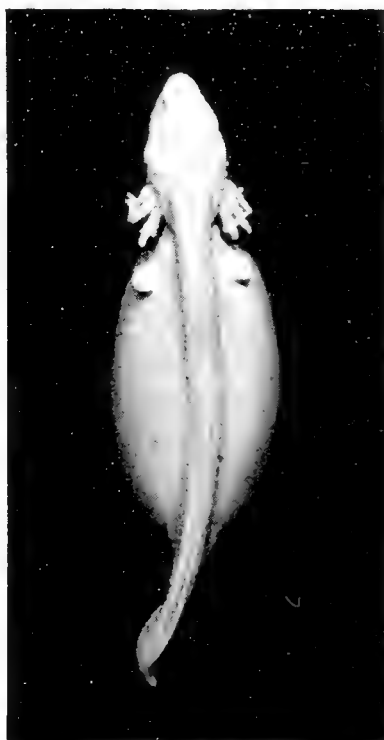
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PLATE 8

EXPLANATION OF PLATES (*Cryptobranchus allegheniensis*)

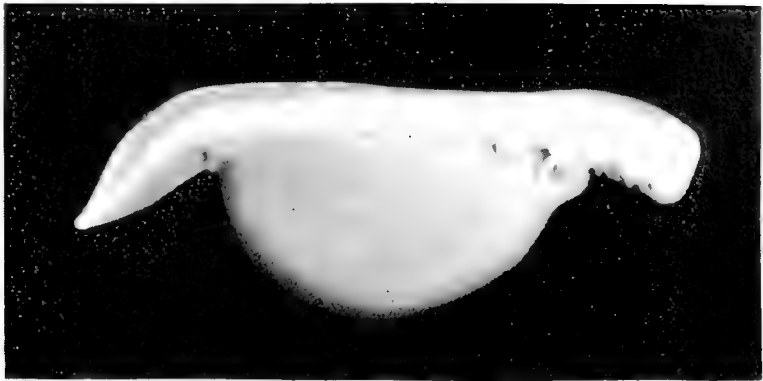
Bichromate-acetic-formalin fixation except for figures 260 and 261 which were made from living specimens anaesthetized with chloretone. $\times 4$.

258 Stage 22. Lateral view.

259 Stage 22.5. Lateral view.

260 Stage 23. Dorsal view of an embryo ready to hatch.

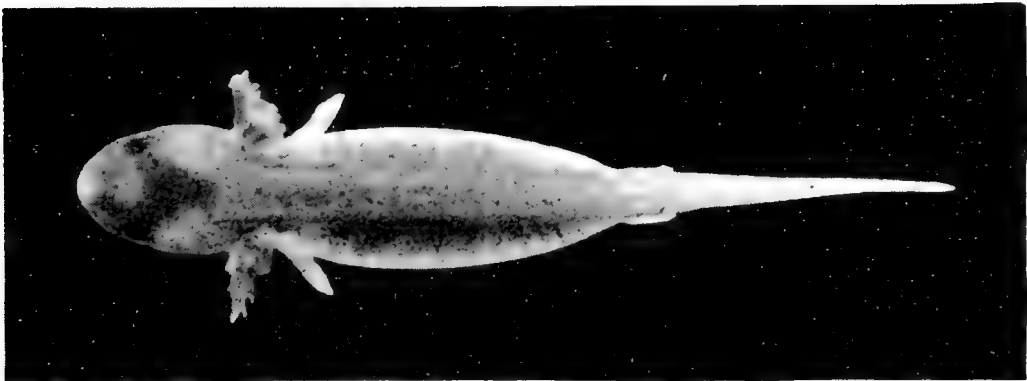
261 Stage 23. Lateral (slightly ventral) view of a newly-hatched larva.



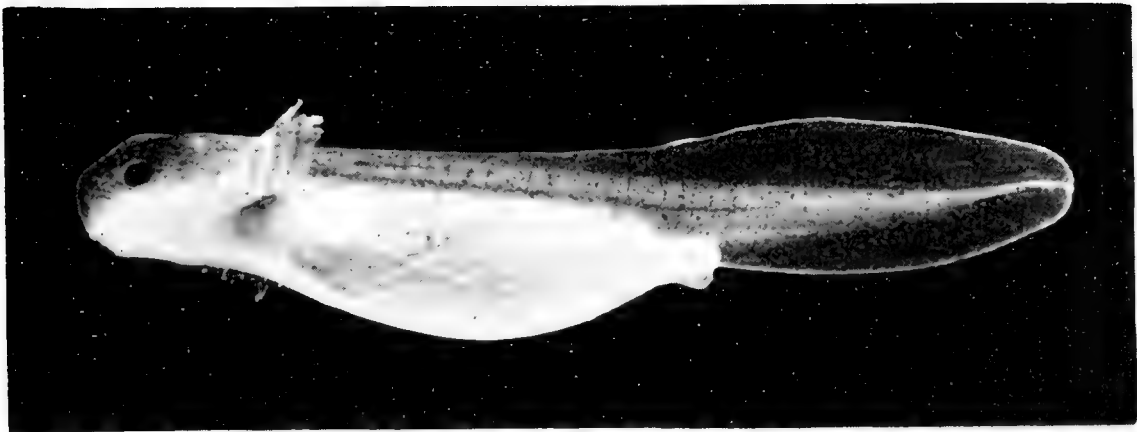
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PLATE 9

EXPLANATION OF FIGURES

(*Cryptobranchus allegheniensis*)

All the figures are natural size.

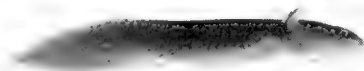
262. Living larvae reared in the laboratory, anaesthetized with chloretone and photographed about five weeks after hatching.

263. Larva reared in the laboratory, killed in bichromate-acetic-formalin about two months after hatching and preserved in formalin. The specimen is slightly shortened through the action of the fixing fluid.

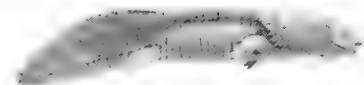
264 and 265. Two views of a larva reared in the laboratory, anaesthetized with chloretone and photographed about ten weeks after hatching.

266. Larva reared in the laboratory, anaesthetized with chloretone and photographed about six months after hatching.

267. Year-old larva captured and fixed in formalin August 27, 1909; a few weeks later it was transferred to alcohol, in which it remained nearly a year before being photographed.



262



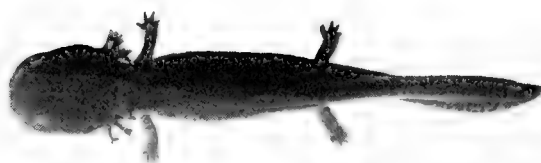
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PLATE 10

EXPLANATION OF FIGURES

(*Necturus maculosus*)

The figures are intended to show in particular the late history of the blastopore. Figures 268 to 276 inclusive were drawn from the living eggs by Prof. Bashford Dean; figures 277 to 279 are from preserved material, and were drawn under the direction of the author by Miss Mabel L. Hedge.

Necturus maculosus





THE STRUCTURE AND METAMORPHOSIS OF THE FORE-GUT OF CORYDALIS CORNUTUS L.

ROBERT MATHESON

From the Entomological Laboratory, Cornell University

TWENTY-NINE FIGURES

Owing to the large amount of work that has been done and is still being done on the metamorphosis of the more specialized insects it has seemed advisable to make a careful survey of some generalized form. It was with this object in view that the present work, though dealing with a restricted region, was undertaken. The writer hopes to continue these studies as time permits.

The present subject was suggested by Prof. W. A. Riley and the work was done under his immediate supervision. To Professors W. A. Riley and J. H. Comstock I wish to extend my thanks for advice and criticism.

STRUCTURE

The fore-gut of *Corydalid* extends as practically a straight, narrow tube from the mouth to the middle of the second abdominal segment where it joins the mid-intestine (fig. 1). There are but two slight enlargements, one at the anterior end, the pharynx, and a more considerable one near the posterior end, the gizzard (fig. 1, *ph.* and *k*). The caudal end projects into the mid-intestine to form a characteristic oesophageal valve. Morphologically it may be divided into five well marked regions: pharynx, oesophagus, gizzard, the region between the gizzard and oesophageal valve, and the oesophageal valve. Along each side of the fore-intestine runs a branch of the visceral nervous system (fig. 1, *n*) while various branches of the tracheal system furnish the necessary air supply.

The tracheal supply of the fore-gut

The distribution of the tracheae to the alimentary canal may be readily seen in figure 1. From the second and third abdominal tracheal gills there arise, just previous to their union with the longitudinal trunks, two comparatively large branches which go directly to the fore-gut. The branch (*b*, fig. 1) from the second tracheal gill first divides into two to five branches which, with their many ramifications, supply the anterior portion of the gizzard and oesophagus. However, there is much variation in the distribution of the tracheal supply in different individuals. In some (fig. 1, *b*) the first branch breaks up into two branches each of which finally divides into a large number of smaller tracheae distributed over the anterior portion of the gizzard while one small branch (*tr*, fig. 2) runs forward along the side of the oesophagus giving off to it numerous small tracheae. This seems to be the more common method of distribution of this trunk. If, however, figure 2 be examined, it is easily seen how greatly modified this may be. Here the first tracheal trunk, *b*, divides into five branches, the posterior one of which is distributed over the caudal portion of the gizzard while the remainder furnish the usual tracheal supply to the oesophagus and gizzard. Even in the same individual the branching and distribution of this trunk varies considerably on the opposite sides.

The second tracheal trunk (*c*) arises from the third abdominal gill (fig. 1, 3 *ab*). It divides just before reaching the intestine into two branches. The posterior branch supplies the ventral caecum as seen on the right side of figure 1. The anterior branch passing forward on the ventral side of the caeca breaks up into five small branches, each of which in turn divides into numerous smaller branches. These furnish the tracheal supply of the posterior portion of the gizzard and that portion of the alimentary canal between it and the mid-gut. There is considerable variation in the branching and distribution of this trunk even on the opposite sides of the same individual. On the left side of figure 1 it may be seen that the large trunk coming from the tracheal gill gives off two branches instead of one to the ventral caecum.

The main trunk passing forward first gives off another small branch which also goes to the caecum. Then it divides into two branches. The anterior one, breaking up into three small branches, supplies the caudal portion of the gizzard. The posterior branch divides into three branches which send numerous small tracheae to the caudal part of the gizzard, the esophageal valve and the ventral caecum.

The third tracheal trunk (fig. 1, *d*) arises at the base of the transverse dorsal trachea of the third abdominal segment. It proceeds forward and divides into two branches, one of which, passing in between the caeca, supplies them with numerous branches and also send tracheae to the fore-gut between the gizzard and mid-gut. The other branch passes to the dorsal side of the dorsal caecum and furnishes it with a tracheal supply. It also sends numerous small branches to the anterior portion of the mid-gut and the region of the fore-gut behind the gizzard.

The fourth tracheal trunk (fig. 1, *e*) arises in a way similar to the second—from the base of the fourth tracheal gill. It passes forwards and divides into two branches. The posterior one supplies the ventral and lateral surfaces of the mid-gut. The anterior one divides into many small branches which supply the anterior and lateral portions of the mid-gut as well as the proximal ends of the caeca and caudal part of the fore-gut.

The fifth tracheal trunk (fig. 1, *g*) arises similarly to the third. Directly at the point of origin or very near it, there is given off a large branch (in some cases two) which goes to the testis. This branching frequently varies on the opposite sides of the same individual. The main branch proceeds forwards and divides just before reaching the digestive tract. The two branches supply the anterior dorsal and lateral portions of the mid-gut as well as the proximal ends of the dorsal caeca and posterior end of the fore-gut.

A sixth trunk (fig. 1, *f*) arises from the fifth tracheal gill. It divides into two branches which supply the posterior end of the mid-gut.

From the photograph (fig. 1) and the above description it will be seen that the gizzard and mid-gut are well supplied with a

tracheal system which is in direct connection with the rich incoming air supply. The oesophagus requires only a very small amount of oxygen and consequently has a poorly developed tracheal system.

The pharynx and its muscles are supplied by a small tracheal branch (fig. 1, *a*) which arises from the exterior longitudinal trachea at its point of entrance into the head. Several other small tracheae from the anterior branches of the main longitudinal trunks supply the cephalic end of the pharynx and mouth-parts.

The pharynx

That portion of the fore-gut, extending from the mouth to near the posterior margin of the head, may be designated the pharynx (fig. 1, *ph*). It is provided with special muscles and, when expanded, is somewhat larger than the oesophagus. On the latero-ventral surface on each side, just behind the sub-oesophageal ganglion, are situated two series of small muscles. These extend in a latero-caudad direction to their points of origin on the posterior margin of the head (fig. 16). The number of these muscles varies, the usual number on each side being from 16 to 19. They lie directly ventrad of the large tracheae which penetrate the head. Directly in front of these lateral-inferior muscles and on the ventral surface there is a series of three to four small muscles on each side of the median line, which pass almost directly ventrad to a short chitinous apodeme near the labial region.

On the dorsal side of the pharynx, directly above the lateral series of muscles, are found two series of small muscles on each side of the median line (fig. 15). The inner series includes five, the outer four, muscles. They are attached on each side of the middle line of the caudal portion of the vertex (fig. 15).

The function of the pharyngeal muscles seems to be that of supporting and enlarging the pharynx during feeding. On opening a freshly etherized specimen it is easy to watch their action. The pharynx in this region is seen gradually to expand, then a wave-like contraction begins at the mouth and passes down the

oesophagus to the gizzard. This contraction is brought about by the circular and longitudinal muscles. On cutting the lateral muscles of the pharynx the expansion preceding the wave-like contraction ceases, although the latter movement continues.

The oesophagus

The oesophagus, except for a gradual increase in size, extends as a straight, cylindrical tube from the pharynx to the middle of the second thoracic segment where it passes into the gizzard (fig. 1, *k*). Viewed externally it presents no important structural characters. The arrangement of the circular and longitudinal muscles, to be discussed more in detail later, can readily be observed in the freshly opened larva (fig. 3). On each side of the oesophagus runs a large nerve (fig. 3, *n*), a branch of the so-called visceral nervous system. Close to each nerve a tracheal branch runs forward as previously described in discussing the respiratory system.

In histological structure the oesophagus, as in all insects so far studied, presents the usual three layers: (1) the epithelium with its intima; (2) the basement membrane lying directly beneath the epithelium; (3) the muscular layer consisting of circular and longitudinal fibers.

The epithelium with its intima and the basement membrane are thrown into numerous irregular folds (fig. 8). At times these folds almost completely close the lumen. The folding allows considerable contraction or distension of the oesophagus and does not, I think, function in the reduction of the food. The resiliency of the intima aids materially in the distension of the oesophagus for the reception of food.

The epithelium consists of a thin layer of flat cells with boundaries very indistinct or lacking. The nuclei are oval in outline, with their long axes usually parallel to the basement membrane (fig. 17). The cytoplasm presents a granular appearance. This part of the fore-gut may secrete a digestive fluid, as Plateau ('74) found in several species of insects examined by him but I found no evidence of it. Its chief function seems to be the for-

mation of a well defined and somewhat extensive intima which lines the interior of the oesophagus and so forms an excellent resilient tube down which the food may pass. A basement membrane is always present.

The muscles, as shown in figure 3, present an arrangement well fitted for the work they perform—the transfer of the food from the pharynx to the gizzard. Next the basement membrane are the longitudinal muscles, which are so arranged that the fibers all run obliquely, crossing each other at a rather acute angle and by this means giving them the effect of two oblique cylinders one woven into the other. These fibers in the region of the gizzard are gathered up into six bundles which pass under the large longitudinal folds (fig. 3) to their points of insertion on the bases of the anterior teeth. Lying directly external to the longitudinal muscles are the circular muscles, small and few, yet with an apparently definite arrangement (figs. 3 and 8, *c.m.*). In the region in front of the gizzard the circular muscles increase rapidly in size and number while from the middle of the gizzard to its caudal end they appear as a dense, thick mass.

From the arrangement of the muscles their action may be readily assumed without actual experimental observation on the living animal. On the reception of food the gradual contraction would force it onward and their relaxation, aided by the resiliency of the intima, would distend the lumen for the intake of more food. Thus there would be a somewhat peristaltic action throughout the length of the oesophagus. This peristaltic action has been observed in the living specimen and described in discussing the pharynx.

The gizzard

The oesophagus passes into the gizzard without any marked constriction. Viewed externally the gizzard presents a barrel-shaped appearance. The only outward means of locating its beginning is by a slight enlargement and an increase in the thickness of the circular muscles. The circular muscles are most greatly increased directly in front of the middle of the gizzard. This increase occurs at the point where the teeth of the gizzard

are most heavily chitinized and meet, almost completely closing the lumen. The lumen can readily be closed by the concerted action of the circular muscles and the longitudinal muscles directly attached to the teeth.

Internally the gizzard presents a remarkable appearance (fig. 4). It is provided with six large, inward-projecting, chitinous ridges and alternating with these are six smaller ridges (fig. 4, *l.r.*, *s.r.*). Each large ridge consists of two prominent teeth, an anterior (*a.t.*, figs. 5 and 6) and a posterior one (*p.t.*), separated by a deep constriction. The anterior tooth projects caudad, the proximal part being somewhat narrow and sharp along its inner edge. It rapidly becomes broader, more strongly chitinized and fits closely over the base of the posterior tooth. Its entire surface is clothed with setae which are longer and more numerous along the posterior border.

Though scarcely distinguishable in surface view the posterior teeth are of two types (compare figs. 5 and 6). They alternate with each other, three of each in a gizzard. The one shown in figure 5 appears in side view somewhat like a pistol, the internal, posterior projecting process answering to the hammer. Viewed posteriorly it presents a broad, somewhat hollowed surface, narrowed on its anterior margin into a sharp tooth projecting caudad. The other type is shown in figure 6 and differs from the first in the lack of the anterior process.

The small longitudinal ridges (figs. 4 and 7, *s.r.*) project between the larger ridges, almost completely filling the intervening spaces. They are not divided into teeth, though at their posterior ends each one presents a broader, setiferous portion projecting caudad.

The epithelium of the gizzard consists of a layer of flattened cells with fairly well defined cell walls. In the tips and sides of the longitudinal ridges the cells are crowded and narrowly elongate. The nuclei are oval to spherical with densely staining granules. The cytoplasm is granular and more or less strongly vacuolate in the cells lining the ridges. The vacuoles are located at the bases of the cells. The longitudinal ridges are filled with a non-cellular tissue (fig. 9, *st*) which is usually designated as a

sort of connective tissue. It is penetrated with tracheae, and tracheal end cells with their nuclei are usually found distributed through it. It does not show any of the characteristics of vertebrate connective tissue when stained by the differential stains used by histologists. It may be analogous to the tissue formed in the developing vitreous body of the vertebrate eye. Here recent researches indicate that it is derived from the prolongations of the cells of the retina, therefore ectodermal in origin. In the case of *Corydalis* this so-called connective tissue stains in the same way as the basement membrane and one can readily trace out into it prolongations of the epithelial cells. The vacuolate condition in the cells near the basement membrane indicates a secretion. If the basement membrane be considered as a product of the epithelial cells I think there is no evidence to show that this supposed connective tissue is not also a secretion of the epithelium. This tissue is widely distributed throughout the intestine and may be referred to under the non-committal term of supporting tissue.

The fore-gut between the gizzard and the oesophageal valve

At its posterior end the gizzard gradually passes into a short, cylindrical tube. The interior of this tube presents internally a thick, chitinous lining without teeth-like processes. Near its posterior end are found four large, chitinous ridges projecting into the lumen (fig. 12). These large ridges extend into the anterior end of the mid-intestine and form part of the oesophageal valve.

Arrangement and action of the muscles

The longitudinal muscles, running in an oblique manner and forming a net-like cylindrical ring around the oesophagus, group themselves into six bundles at the beginning of the gizzard (fig. 3). These six bundles pass respectively under the six large longitudinal folds and attach themselves to the bases of the anterior teeth. At a point just cephalad of that where these muscles are attached, there arise six other longitudinal muscles which run caudad and are inserted on the anterior elongated bases of the posterior teeth. The fibers of these two sets of muscles form a

mesh-like arrangement as they pass to their respective points of insertion and origin on the bases of the anterior teeth.

Behind the anterior bases of the posterior teeth there are no longitudinal muscles till near the end of the gizzard. The longitudinal muscles found caudad of this point are the terminating portions of those extending forward from the mid-gut and lie externally with relation to the circular muscles. When found underlying the circular muscles they are passing to their points of insertion.

The circular muscles of the gizzard are much increased in the posterior half where the powerful teeth are located. Behind the gizzard they are small and few in number till we reach the oesophageal-valve region. This occurs at the beginning of the four longitudinal ridges which almost completely close the lumen leading into the mid-gut. Also at this point the longitudinal muscles from the mid-gut pass in large numbers between the caeca and are inserted on the chitinous ridges, the ridges alternating with the caeca.

From the arrangement and position of the muscles of the gizzard their action seems apparent. By the contraction of the circular muscles the lumen can be completely closed, thus exercising a crushing and straining action on the enclosed food. The longitudinal muscles, contracting and relaxing at the same time as the circular muscles, would give to the large teeth a grinding action on each other effecting a vigorous trituration of the food. The relaxation of all the muscles combined with the resiliency of the intima and the onward motion of any food in the oesophagus would open the gizzard allowing the entrance of more food. Thus there would be a fairly rhythmic action of the gizzard—contraction and gradual distension following each other.

The action of the muscles of the oesophageal valve is clear. The circular muscles by their contraction can readily close the lumen, preventing the passage of unbroken food particles or, in connection with the oesophageal valve, regurgitation from the mid-gut; the longitudinal muscles in turn, aided by the resiliency of the intima, open the lumen thus allowing the onward passage of food from the gizzard.

The oesophageal valve

In *Corydalis* the oesophageal valve is not so marked as that found in many other insects. It is formed by the invagination of the fore- into the mid-gut. This invagination is short and is lined internally by a thick intima which is folded into four large, tooth-like ridges (fig. 12). Externally to the basement membrane lie the circular muscles. Outside of these are found longitudinal muscles, continuations forward of the mid-gut fibers. They group themselves as they pass between the caeca and most of them are attached to the four chitinous ridges. Others continue forward and are attached at various points behind the gizzard. These longitudinal muscles, besides their function of opening the lumen, undoubtedly serve to hold the valve in place and prevent its evagination by the pushing back of any congested food in the mid-gut. The epithelium lining the valve is of the ordinary type found in the fore-gut except for a group of cells located at the point of union between the fore- and mid-gut epithelium (fig. 10, *g.e.*). These cells clearly present a glandular appearance but stand in marked contrast to the glandular epithelium of the mid-gut (fig. 10, *ep.*). What the particular function of this group of cells is would be difficult to conjecture as I do not find any peritrophic membrane and they do not show any of the characteristics of an imaginal ring as described for so many insects.

METAMORPHOSIS OF THE FORE-GUT

Pupation

The mature larvae leave the water in the latter part of May or June and pupate in cavities under stones near the stream. Davis ('03) found that the pupal life lasted from seven to fourteen days, the average being nine days. The larvae from which my material was obtained were received about the 23d of May, 1910, and were placed in a dish with a small amount of water. The dish was then placed in a box of moist earth over the surface of which were placed several flat stones. The larvae immediately crawled out

of the water and began burrowing under the stones. In a short time many of them had completed their pupal chambers.

The time spent in the pupal chamber varies from one day to two weeks (Davis '03). In my specimens the first larva pupated on June 5, twelve days after the formation of the pupal chamber. The remainder pupated at various intervals up to the 12th or 13th of June. Specimens were taken at various intervals, from the time the pupal chambers were formed until the emergence of the adults. They were killed with chloroform and the alimentary canal removed under normal salt solution. Various fixing agents were used, all with good results.

Shortly after the formation of their burrows the larvae become sluggish. The changes in the intestine which may be observed by simple dissection are worthy of mention. At first the greater part of the fore-gut is filled with a dense, blackish fluid, evidently undigested food material, as this condition is found in the larvae while feeding and growing. Very early the fore-gut becomes emptied and remains empty throughout the entire prepupal and pupal period except at the posterior end where some of the congested material, formed by the breaking down of the epithelium of the mid-gut is found. Figure 11 shows the condition of the fore- and mid-guts at the time of pupation. In this case the intima of the gizzard was not shed at the time of the molt, the only example of this found. It remained lodged at the anterior end of the oesophagus.

The histological changes undergone by the various systems of organs during metamorphosis have not been fully investigated except in a few scattered species of insects. Even here the changes observed are so complex that nearly every worker has offered a different interpretation of the phenomena. Owing to this wide divergence in the interpretation of the histological changes it will first be necessary to give a hasty review of the work already done before taking up in detail my observations on *Corydalis*. In this review I shall confine myself to the work that has been done on the fore-gut.

The epithelium of the fore-gut

Weismann ('64), the pioneer worker on the post-embryonic development of insects, thought that the entire fore-gut in the Muscidae was broken down during metamorphosis by a sort of fatty degeneration, the degenerated epithelium falling into the lumen of the intestine and forming the yellow body. Weismann did not solve the method of regeneration of the fore-gut, though he thought the entire intestine developed from the cellular mass formed by the broken down larval tissues. This, he believed, took place by a sort of free-cell formation; the particles isolating themselves, forming enveloping membranes, losing their fatty granulations, and each acquiring a nucleus. To these formations Weismann gave the name of 'Kornchenkügeln.' From such cells the imaginal intestine developed. Later Kowalevsky ('87) showed that the 'Kornchenkügeln' are only phagocytic leucocytes which have been swollen by the engulfment of large quantities of larval tissues and they take no part in the formation of the imaginal tissues.

In *Corethra* according to Weismann ('66), the digestive epithelium passes directly over to the imago without undergoing any important modifications.

Ganin ('76) states that:

The fore-gut of the larva together with all its appendages are completely broken down (*Anthomyia*, *Musca*, *Scatophaga*, *Eristalis*, *Stratiomyia*, *Formica*, *Myrmica*, *Lithocolletis*, *Chrysomela*, and *Tenebrio*). The food for the formation of the imaginal epithelium is furnished by the broken down larval cells. The products of the broken down epithelium float freely in a thick fluid between the intima and the basement membrane. If we observe closely the histological structure of the fore-gut (at the end of the so-called proventriculus—oesophageal valve) we find in each mature larva, at the point where the mid-gut epithelium ends, a narrow strip of peculiar tissue which is sharply differentiated from the rest. This narrow strip consists of very small, round, transparent cells which possess all the characteristics of young, embryonic tissues. The later developmental stages demonstrate that from this small group of cells is built up the entire imaginal epithelium although I was not able to trace the re-formation to the mouth.

Kowalevsky ('87) is the first worker to give a complete and detailed account of the metamorphosis of the fore-gut. In the

blow-fly (*Musca vomitoria*) he found an imaginal ring situated at the point of union of the fore- and mid-gut epithelium. This peculiar ring of cells was present in the youngest larva examined. During metamorphosis the fore-gut becomes shortened and the larval epithelium is replaced by the active proliferation of the cells forming the imaginal ring. These proliferating cells form the entire imaginal epithelium except the most cephalic part which he considered as consisting of transformed larval epithelium. The cast-off larval epithelium is destroyed by phagocytes.

Van Rees ('89) in studying the same insect as Kowalevsky, comes to essentially the same conclusions and verified in a remarkable way the results of the Russian naturalist.

Rengel ('96) in his studies on *Tenebrio molitor* dismisses the fore-gut with the statement that it retains its relative length and shape through the life of the insect.

Mobusez ('97) comes to the conclusion that not only the mid-gut but also the fore- and hind-guts undergo deep-seated changes during the larval molts and at pupation.

Karawaiew ('98) finds no imaginal ring present in the fore-gut of *Lasius flavus*. During pupation the lumen of the caudal portion of the fore-gut becomes greatly reduced. The epithelium, which near the middle of the oesophagus consists of a single layer of cells, becomes several layers thick at the caudal portion. He was unable to explain this condition. From this region the crop and gizzard develop through growth and differentiation. In general he found but slight changes in the epithelium except at the caudal end where the transformations are not fully explained.

Vesron ('98) as the result of his studies on the metamorphosis of the silk-worm adopts a new interpretation of the function of the imaginal ring. He regards it simply as a center of growth throughout the life of the insect. It originates in the ectoderm from the germinal band and forms the point of invagination of the stomodeum. During the larval life this imaginal ring proliferates at each successive molt, thereby increasing the intestinal epithelium. At the time of pupation it again proliferates in exactly the same way as at the larval molts. The cells thus arising from the imaginal ring unite with those previously formed, with-

out destroying or replacing them in any way whatever. Thus he considers the epithelium to pass over to the imago, though somewhat modified to adapt it to the changed conditions. As the epithelium is sharply differentiated from the hypodermis he excludes any possibility of regeneration from imaginal buccal discs.

Karawaiew ('99) in his studies on *Anobium paniceum* (Coleoptera) finds but slight changes in the fore-gut during metamorphosis. The imaginal epithelium develops directly from the larval without any apparent changes. In this respect this beetle exhibits much similarity to the ants.

Anglas ('00) finds in the bee and the wasp a well-marked zone of growth between the fore- and mid-guts. He does not regard this as an imaginal ring but more like the condition found by Karawaiew in *Lasius flavus*. By the proliferation of the cells of this growth area the greater part of the imaginal epithelium is formed. The proliferating cells invade and engulf the larval cells. The anterior part of the larval oesophagus becomes that of the adult. He finds it difficult to distinguish the point of union of the part formed by the cells from the growth zone and that formed by the transformed larval epithelium. One passes insensibly into the other.

Deegener ('00) finds an imaginal ring present in the fore-gut of *Hydrophilus*. During the prepupal period this imaginal ring rapidly increases by mitotic divisions of the cells, while the larval epithelium is discharged into the lumen where is found a considerable number of 'Kornchenkügeln.' During the pupal period the imaginal epithelium is formed into a cylindrical tube within which are found remnants of the larval cells and Kornchenkügeln. Later Deegener ('04) finds he was in error regarding the presence of Kornchenkügeln within the lumen and he states that phagocytes do not take any part in the breaking down of the larval epithelium.

Perez ('02) describes in *Formica* an imaginal ring for the fore-gut, situated as in the *Muscidae*. During the prepupal period the cells of this ring actively divide and the epithelium of the oesophageal valve is discharged into the lumen. By the continued proliferation of the imaginal ring the fore-gut is elongated and

the larval epithelium is forced cephalad to become the imaginal oesophagus.

Vaney ('02), in his studies on dipterous larvae, finds imaginal rings present in the following;—*Chironomus*, *Anthomyia*, *Stratiomyia*, *Tanypus* and *Gastrophilus*. In *Stratiomyia* he also found in front of the oesophagus a small buccal disc, while in *Gastrophilus* there is one just caudad of the pharynx. Only in *Gastrophilus* did he study the metamorphosis of the fore-gut. There was degeneration of the larval epithelium in situ and its destruction by phagocytes. The imaginal epithelium arises exclusively from the imaginal ring and the post-buccal disc.

Deegener ('04), in his studies on the metamorphosis of *Cybister roeselii*, records a prominent imaginal ring situated at the point of union of the fore- and mid-gut epithelium. He divides the metamorphosis into two distinct periods;—(1) the shedding of the larval intima and the formation of the pupal epithelium; (2) the molting of the pupal intima and the rebuilding of the imaginal epithelium. The first period is completed about twenty-four hours after pupation. The epithelium of the anterior portion of the oesophagus becomes that of the pupa, with but slight changes in the nuclear chromatin and cellular cytoplasm. The epithelium of the posterior portion breaks down and is forced into the lumen by the rapid forward growth of the imaginal ring. The degenerated larval cells form a deeply staining mass lying between the molted larval intima and the developing pupal epithelium. The imaginal ring cells divide mitotically with extreme rapidity. The spindles are always located in the plasma layer next the lumen, their long axes parallel to that of the intestine. Numerous degenerating nuclei are observed directly in front of the imaginal ring. Deegener thinks that the larval cells fail in the struggle with the imaginal, become broken down and absorbed by the more actively growing embryonic cells.

The pupal epithelium is completed about the end of the third day after pupation. Then immediately begins the formation of the definitive fore-gut. The posterior part, through growth and a great increase by the division of the imaginal ring cells, becomes differentiated into the crop and gizzard of the adult. The ante-

rior part, with but slight changes in its nuclear and cytoplasmic contents, becomes that of the imago. Deegener states that the intima, both pupal and imaginal, is formed by a direct transformation of the inner plasma layer of the epithelium.

Verson ('05) presents the detailed results of his work on *Bombyx mori*. In respect to the fore-gut he affirms the conclusions given in his previous paper (1898).

Thompson ('05) finds that in *Culex* the oesophageal epithelium undergoes no metamorphosis. It is handed over intact to the imago with only an increase in the number of its component cells.

Van Leeuwen ('07) in his studies on *Isosoma graminicola* finds no imaginal ring present, though he states that the posterior part consists of cells, differing from the rest and containing many small nuclei. It is here that the most important changes during metamorphosis occur. There is a marked lengthening of the caudal part of the fore-gut during the pupal period by mitotic cell division. The anterior division degenerates in situ and is replaced by a hypodermal invagination. In the caudal region are developed the sucking stomach and gizzard.

Lubben ('07) finds but slight changes in the fore-gut of *Trichoptera* during metamorphosis. There is no imaginal ring present (in the sense of Kowalevsky) though the entire oesophageal valve might be considered one, as he states Deegener ('04) does for the beetle intestine. In this portion there is considerable cell increase. The main changes are the flattening of the pupal epithelium, a considerable increase in length and the formation of the characteristic imaginal gizzard. He doubts whether the intestine functions in the adult.

Russ ('08) in his studies on *Anabolia laevis*, one of the *Trichoptera*, finds a well marked imaginal ring present, which consists of tall, cylindrical cells heaped upon one another in several layers. The anterior part of the oesophagus, except for slight changes, becomes that of the imago. Except in the very mouth region he finds in this part a partial renovation through the divisions of the larval cells. In the caudal portion the larval epithelium degenerates, being absorbed and replaced by the actively growing embryonic cells. The pupal epithelium of the entire fore-gut

consists of tall cylindrical cells. These become much flattened in the adult and there is consequently a great enlargement of the lumen.

Deegener ('08) investigated the metamorphosis of the intestine of *Malacosoma castrensis* (Lepidoptera), which has a well marked imaginal ring for the fore-gut. As in *Cybister* he divides the time of transformation into two distinct periods;—the shedding of the larval intima and the formation of the pupal epithelium; the shedding of the pupal intima and the formation of the imaginal epithelium. The first period is completed at the end of the first pupal day. The pupal epithelium is identical with that of the larva except for a slight transformation of the cellular contents. He does not find any activity on the part of the imaginal ring, although in its neighborhood there may be seen a few degenerating nuclei during the pupal period. About the end of the third day after pupation the pupal intima is molted and the formation of the imaginal epithelium begins. This is completed between the fifth and sixth days of the pupal life. The pupal epithelial cells, except for a slight reduction in size and rearrangement of their chromatin material, pass over directly into the imaginal epithelium.

It might be further noted here that Deegener considers the basement membrane as a temporary structure, appearing only while a well developed intima is lacking or to protect the epithelium from the severe contractions of the surrounding muscles.

Perez ('10) in his extensive studies on the metamorphosis of the Muscidae confirms and extends the results of Kowalevsky ('87) and Van Rees ('89) in regard to the post-embryonic development of the fore-gut.

THE EPITHELIUM IN CORYDALIS DURING METAMORPHOSIS

The prepupal period

The characteristics of the larval epithelium have been fully discussed and illustrated in the first part of this paper. We shall now examine the changes undergone during the prepupal period up to the time of the last larval molt. The first changes appear

in the oesophageal valve region where the cells become elongated and narrowed and the cell walls more distinct. The nuclei are more crowded and more chromatic. The cytoplasm appears more granular and shows a sharper differential staining reaction. The intima shows little change except for a slight granular appearance near the epithelial cells. It has not yet become separated from the epithelium. These general observations hold for the entire fore-gut at an early prepupal stage. But in the anterior portion the cells do not elongate so much nor do the cell walls become so sharply differentiated. There is no imaginal ring present and the peculiar glandular cells found between the fore- and mid-guts show no activity throughout the entire prepupal and pupal periods.

The absence of an imaginal ring is in marked contrast to the condition described by several authors for a number of other insects. In all the dipterous forms so far carefully studied an imaginal ring is recorded (Ganin '76, Kowalevsky '87, Van Rees '89, Vaney '02 and Perez '10). It is recorded as present in the Hymenoptera by Ganin ('76) for *Formica* and *Myrmica*, and Perez ('02) for *Formica*. Karawaiew ('98) notes its absence in *Lasius flavus*, Anglas ('00) in the bee and wasp, and Van Leeuwen ('07) in *Isosoma graminicola*. In the Coleoptera Ganin ('76) finds it in *Chrysomela* and *Tenebrio*, Deegener ('00) and ('04) in *Hydrophilus* and *Cybister*. Karawaiew ('99) notes its absence in *Anobium paniceum*. Ganin ('76), Verson ('98) and Deegener ('08) record its presence in Lepidoptera (*Lithocolletis*, *Bombyx mori* and *Malacosoma castrensis*). In the Trichoptera Lubben ('67) regards the imaginal ring as absent while Russ ('08) records a prominent one for *Anabolia laevis*.

In a slightly older prepupa than that just described the intima becomes completely separated from the epithelium. The inner surfaces of the cells show in many places small, protoplasmic projections as if they had been drawn out of the intima. These are not shed into the lumen but later become flattened down. There are no signs of degeneration. The nuclei show their characteristic staining reactions while the cytoplasm remains granular (fig. 19). The intima, however, shows considerable change.

In the larva not preparing to pupate (fig. 17) the narrow, inner, chitinized layer stains densely black with iron-haematoxylin while the remainder usually shows a very characteristic laminate appearance. In the prepupa, at the time of molting of the larval intima, the black area is much increased and the inner layer loses somewhat of its laminate structure. It has a broken down appearance, often reticulate, the loose intertwining strands filling the space between the epithelium and intima (fig. 19). The basement membrane is sharply differentiated throughout the entire prepupal period.

Changes at time of pupation

The external changes in size, shape, et cetera, may be seen in figure 11. In this pupa the intima of the gizzard was not discharged at the molt but remained lodged at the anterior end of the oesophagus. The fore-gut is now a long, cylindrical tube practically empty, while the mid-gut is greatly expanded by a dense, yellowish fluid. None of this fluid extends into the fore-gut except at its posterior end where the nearly evaginated oesophageal valve fails to form a closing bridge. A short distance cephalad, however, the fore-gut is completely closed.

As *Corydalis* larvae, under similar conditions, vary so much individually in the time spent in the pupal chamber before pupation, as well as the pupae in the time occupied before the emergence of the adult, I have found it impossible to follow the changes according to the time element. The changes to be described here as occurring at the time of pupation cannot be said to always occur at this period, since my experience shows that the individual larvae pupate when different histological changes are in progress. I shall, therefore, present in as connected and as logical a way as possible the changes that occur throughout pupation up to the time of the emergence of the adult, without special reference to the particular time at which these changes are to be observed.

With the shedding of the larval intima the oesophageal valve becomes partially evaginated and appears in cross-section as a

large, cylindrical tube with a somewhat flattened epithelium in its anterior portion and distended with fluid from the mid-gut. In longitudinal section the valve is never seen to form an open cylindrical tube, but the walls, which in the larva project into the mid-gut, now form a wide ring projecting at right angles to the long axis of the alimentary canal and almost closing the lumen. The epithelium of the posterior portion consists of rather tall, narrow, cylindrical cells with well defined walls. The nuclei are large and chromatic, are situated near the bases of the cells, and do not show any indication of degeneration. The cytoplasm is vacuolate and granular, the vacuoles being located near the tips of the cells (fig. 21, *v*). At this stage no epithelial cells were found undergoing division. A well defined basement membrane is present and a pupal intima is formed though it stains but slightly in eosin and not at all with iron-haematoxylin.

The tall, glandular-appearing cells, situated in the larva at the juncture of the fore- and mid-gut epithelium, have become greatly reduced in size and show no signs of secretory activity. They have become narrow, cylindrical cells like the rest of the fore-gut epithelium.

Throughout the entire fore-gut the epithelium is thrown into longitudinal folds, but the folds are much reduced as compared with those of the larval oesophagus. The lumen is closed a short distance in front of the oesophageal valve, but appears again slightly cephalad and continues as a small tube almost closed by the longitudinal folds. The circular muscles are in a state of active contraction.

Changes from the time of pupation to the shedding of the pupal intima

The next stage which requires special attention is that from the time of pupation to the shedding of the pupal intima. The time this occupies is dependent largely upon the individuals examined, but requires two to three days. Some time after pupation in the region formerly occupied by the larval gizzard, the epithelium, which consists of greatly crowded cells, shows many nuclei apparently undergoing chromotolysis (figs. 13 and 14, *x*).

The chromolytic drops are located near the basement membrane, and it is difficult at times to decide whether one is observing a degenerating nucleus or one that is massing its chromatin preparatory to division. But, judging from the position of these deeply staining masses and the work of previous authors, it would seem conclusive that these are actually degenerating nuclei. None of the degenerating nuclei or cellular contents are discharged into the lumen but are undoubtedly absorbed by the neighboring cells. In no case have I observed any part of a cell or its contents discharged into the lumen or forced out through the basement membrane. Cephalad or caudad of this region very few degenerating nuclei are found.

The epithelial cells show decided secretory activity. The nuclei are prominent, chromatic and located near the bases of the cells (fig. 21). The cytoplasm is granular and almost filled with large vacuoles (figs. 13 and 21). At this period also many nuclei in the gizzard region are undergoing mitotic division. The cell preparing to divide becomes large, migrates to the inner surface, retaining only a very narrow connection with the basement membrane, and at the same time possessing a well marked cell wall (figs. 27 and 28). Very few mitotic figures are found either in the anterior oesophageal region or near the oesophageal valve. The epithelium is still thrown into many longitudinal folds which at this stage practically close the lumen. There is also a considerable reduction in the diameter of the canal. The changes described above are usually found in pupae from two to three days old.

At a slightly later period further changes may be observed. There is a constant decrease in the size of the intestine and a marked reduction of the longitudinal folds. The epithelial cells are gradually being reduced in size, due to the active secretion of a very large quantity of pupal intima. The vacuolate condition is also being reduced. Many nuclei throughout nearly the entire fore-gut are undergoing division and many are degenerating. The cellular contents possess the same characteristic appearance and give the same staining reactions. Here and there the pupal intima is separated from the underlying epithelium (fig. 29).

The shedding of the pupal intima

This occurs three to four days after pupation, and the changes that have been going on up to this time become more intensive. The epithelial cells retain their characteristic appearance. The number of degenerating cells is rapidly decreasing, while there is a marked increase in the number undergoing division. In any cross section one can count from four to seven nuclei undergoing mitosis. The dividing nuclei are not so numerous in the anterior or posterior portions. The pupal intima now lies free within the lumen. Between the cast intima and the epithelium one finds a somewhat granular secretion filling up this area and so closing the lumen (fig. 26, *g.m.*). Owing to their great secretory activity the cells are being rapidly reduced in size; the vacuoles, now nearly all at the tips of the cells, are smaller or frequently lacking; the longitudinal folds are lacking except near the posterior end where numerous small folds are being formed; the intestine is somewhat larger. The oesophageal valve is now reformed as it will appear in the imago except for slight cellular changes.

The beginnings of the imaginal intima now show their early appearance. In figure 22, one observes near the inner surface of the cells a row of very distinct, sharply staining, black dots, underlying a granular layer which does not show very clearly in the photograph. This is shown much better in figure 29, *r*, from a pupa only forty-eight hours old, though it is not commonly seen so early. This condition agrees with what Deegener ('08) considers as the origin of the intima in the developing fore-gut of *Malacosoma castrensis*. As the imaginal intima appears in this place one seems forced to conclude that it is formed not only by secretion but also by a direct transformation of the cells themselves.

Formation of the imaginal epithelium

The changes that occur after the shedding of the pupal intima are not very considerable. The oesophageal valve remains practically the same as in the four-day-old pupa. In the region directly cephalad of the valve the formation of longitudinal ridges continues to a marked degree. This occupies only a short distance,

as the major part of the fore-gut consists of a smooth cylindrical tube. Throughout the entire epithelium there is a gradual reduction in the height of the cells and an increase in the diameter of the intestine. From the time of the shedding of the pupal intima till about the ninth day there occurs a constant increase of cells by mitotic division, and degenerating nuclei are found in gradually reducing numbers. With the definitive formation of the imaginal intima one does not find any more dividing or degenerating nuclei.

Finally, in a pupa ten days old the epithelium appears in practically the same form as in the adult. It is arranged in numerous, small, longitudinal folds throughout the greater part of the oesophagus. In the posterior portion this folding is very marked. It consists of flattened cells with granular cytoplasm and large nuclei. The vacuolate condition, which was so marked in the early stages, has now disappeared. The great reduction in the size of the cells is due both to the secretion of a large quantity of granular substance which lies between the shed pupal and the forming imaginal intima, and to the increase in the diameter of the canal. The cellular walls are very indistinct or lacking. The imaginal intima appears as a narrow, light area and is now well formed. A basement membrane is present throughout the entire pupal period. At this stage no degenerating nuclei or any undergoing division are found.

Shortly after the loosening of the pupal intima there occurs a slight outpocketing of the walls of the oesophagus a short distance in front of the oesophageal valve. This has been described and figured by Leidy ('48) who designated it the pupal crop and notes its resemblance to the sucking stomach of Lepidoptera. During its formation the epithelial cells in this region undergo rapid and extensive mitotic divisions. The epithelium lining this outpocketing is identical with that of the oesophagus. What its function may be is doubtful. It passes over to the imago as a small appendage of the posterior portion of the oesophagus.

Cell division

The phenomenon of cell division in the epithelium of the fore-gut is very characteristic. The cell, preparatory to division,

enlarges and the greater portion migrates towards the distal surface (figs. 27 and 28, *m.*). Each cell, however, retains a narrow protoplasmic strand connecting it with the basement membrane (fig. 28, *st*). A cell wall is always present and well defined. The nuclear spindle is nearly always parallel to the long axis of the intestine and is placed at the inner side of a large vacuole. The presence of a vacuole in every dividing cell, together with its staining reaction, clearly prove that these cells belong to the ordinary larval epithelium and are not tracheal cells which have migrated through the basement membrane, as Anglas ('04) thinks is the origin of the replacement cells in the Hymenoptera. The only method of cellular increase in the fore-gut epithelium is by indirect divisions. Shortly after pupation one finds scattered cells undergoing division in the larval gizzard region. The number gradually increases, reaching its maximum shortly after the shedding of the pupal intima. Then follows a gradual reduction, till division ceases just before the definitive formation of the imaginal intima. The cellular increase is greatest in the larval gizzard region.

HISTOLYSIS AND HISTOGENESIS OF THE INTESTINAL MUSCLES

Histolysis

Before attempting a discussion of the histolysis of the muscles in *Corydalis* it may be well to present as briefly as possible the current interpretations of so complex a phenomenon. At present there are several theories, each apparently well founded upon observed facts:

1. That of the leucocytic phagocytosis of Kowalevsky, Van Rees, and Mercier. According to these workers the muscles are attacked, broken down and engulfed by leucocytes before any chemical changes visible under the microscope have taken place within the muscle substance. Digestion of the débris occurs within the leucocytes.

2. That of the modified leucocytic phagocytosis of Loos, Bataillon, and Mercier for Amphibia; Deegener, Vaney, Verson, and others for insects. According to this theory there is, first, a

chemical and physical change in the muscle substance before leucocytic intervention. The activity of the leucocytes may be great (Mercier, Vaney, etc.) or very unimportant (Deegener, Karawaiew, etc.). The preceding chemical changes may be slight or very marked.

3. The lyocytic hypothesis of Anglas ('00). According to this view there is no engulfing of sarcoytes but an extracellular digestion by means of diastases excreted by the leucocytes. The tissues thus dissolved are absorbed by the leucocytes. This process Anglas designates 'lyocytosis.'

4. The auto-phagocytic theory of Metschnikoff for Amphibia, De Bruyne and Russ for insects. According to this theory some of the muscle nuclei surround themselves by protoplasm and constitute true phagocytes (sarcoclastes or myoclastes of De Bruyne). De Bruyne found this condition of the degenerating muscles of *Musca vomitoria* while Metschnikoff regards it as the normal process in the absorption of the tail in *Anura*. Russ ('08) adopts this interpretation for the degeneration of the muscles in *Anabolia laevis*, one of the Trichoptera.

5. The purely chemical and physical hypothesis of Korotneff. In a Tineid larva he finds the muscles are entirely broken down by a chemical liquefying process without any intervention of phagocytes. Karawaiew adopts this view for the ants (*Lasius niger*), Terre ('99) for the bee and Deegener ('04) for *Cybister*. Korotneff admits a true phagocytic activity where the metamorphosis is extremely rapid, as in so many Diptera. He advocates the hypothesis that where metamorphosis continues for a considerable time there is no phagocytic intervention, but on the other hand phagocytes play the all-important rôle where the changes are rapid.

6. The theory of Berlese. According to this worker the first change to appear is the separation of the sarcolemma and the nucleus from the fibrillar part. This he calls myolysis. The fibers are chemically dissolved whereas the nucleus with its plasma survives—it is the living part of the cell capable of regenerating new contractile fibers. The dissolution of the fibers (fibriolysis) is probably caused by the extravasation of the intestinal contents

at the end of the larval life. The partially broken down fibers (sarcolytes) are taken up by means of the protoplasmic expansions of the amoebocytes (leucocytes of other authors) and carried by them to all parts of the body where regeneration is in progress, furnishing the necessary nutrition. These are the so-called 'Korchenkügeln,' by Berlese designated sarcolytocytes. The sarcolytocytes are found only in the more highly specialized insects. Within the sarcolytocytes no digestion takes place, they simply act as carriers of the débris. The muscle nuclei, with their cytoplasm, become independent cells. The nuclei fragment, each forming a mass within its own enveloping membrane (sarcocyte). These sarcocytes play an important rôle in the histogenesis of the imaginal muscles.

The metamorphosis of the intestinal muscles of insects has been investigated by several workers. In nearly all the dipterous forms they are destroyed by phagocytes without a previous chemical change visible under the microscope (Kowalevsky, Van Rees and Perez). Vaney ('02) thinks there is a chemical change within the muscle before the leucocytes become active (in *Gastrophilus*). Thompson ('05) finds no phagocytosis in *Culex*. In all the Coleoptera so far studied there has not been found any phagocytic activity, the muscles apparently liquefying in place (Karawaiew '99, Breed '03 and Deegener '04). In the Hymenoptera Karawaiew ('98) and Van Leeuwen ('07) hold to the chemical and physical hypothesis, while Perez ('02) finds phagocytosis in *Formica*. In the Lepidoptera Korotneff denies any phagocytosis while Verson ('05) and Deegener ('08) find phagocytic activity following a chemical dissolution. In the Trichoptera Russ ('08) thinks there is present a form of auto-phagocytosis though he is doubtful of his interpretation of the process in the species (*Anabolia laevis*) studied.

In *Corydalis* the fore-gut possesses a well developed muscular system. The fibers are all cross striated, the striations standing out very clearly. During the prepupal period there are no visible changes until about the time of the loosening of the larval intima. At this time the muscles become strongly contracted, as is shown by the frequent crenulate condition of the sarcolemma and the

obscuration of the finer discs, Krause's membrane and Hensen's disc. The nuclei are more chromatic, large and more prominent. This strikes one forcibly when comparing sections of the muscles of the larva and the prepupa (figs. 17 and 19). The sarcoplasm is also more prominent, though undoubtedly this is due to the great contractions of the fibers. I do not find any marked differences in the staining reactions of the fibers. Scattered among the muscles are found a considerable number of leucocytes, which, however, show no signs of special activity. They are more numerous in the posterior region, particularly near the oesophageal valve. During the larval life it is rare to find any leucocytes in the neighborhood of the fore-gut.

At the time of pupation the changes above outlined become more marked. The cross striations are beginning to disappear, while the outer portions of the circular muscles show in many places a granulose structure. The cross striations are affected earliest in the neighborhood of the nuclei and show first near the anterior end of the oesophagus. As to why this degeneration should first appear around the nuclei seems difficult to explain except on the basis of the location of the greatest cytoplasmic activity near the nuclei. This does not indicate auto-phagocytosis in any sense of that word. It simply indicates that the chemical activity is greatest where the cytoplasm is greatest and the liquefying process proceeds outward from the nuclei as centers. This, I think, is borne out by the facts shown in the degeneration in the muscles of *Corydalis*.

The sarcoplasm is constantly increasing in amount while the nuclei are large and prominent (fig. 20). In cross-sections the areas of Cohnheim do not stand out so sharply. As yet there is no noticeable change in the fibrillar structure except near the nuclei. Leucocytes are distributed somewhat sparingly around and among the muscles, but they do not show any activity in the destruction of them.

At a slightly later period the cross striations have almost completely disappeared except near the posterior end of the fore-gut, while here and there scattered strands show a faint cross striate condition. The greater part of the circular muscles have

not only lost their cross striations but their fibrillar part as well (fig. 21). The densely staining portion in figure 21 is the remnants of the fibrillae undergoing degeneration. The muscular layer now appears as a granular, cellular mass, separated by the undissolved sarcolemma and remnants of the supporting tissue. The nuclei are not so deeply staining nor so prominent as at the time of pupation. Surrounding the fore-gut are found numerous leucocytes which are undoubtedly taking up some of the broken down tissues, as many are seen to contain deeply staining granules. The above described changes are completed about twenty-four to forty-eight hours after pupation.

The process of liquefaction of the fibrillae continues till about the time of the shedding of the pupal intima. At this time the muscular layers have the appearance shown in figure 22. Here and there are found nuclei undergoing chromotolysis while some are dividing mitotically. The fibrillar structure is practically lacking, while the nuclei and surrounding cytoplasm appear somewhat rejuvenated. Many leucocytes are found surrounding the muscular layers and are absorbing portions of the chromolytic drops as may be seen from their contents (fig. 22, *l.*). We have now reached the end of the histolytic process. The muscular tissue is not destroyed by phagocytic leucocytes but seems to liquefy in place, the process beginning about the nuclei as centers. Undoubtedly, as previously pointed out, some of the débris is engulfed by the leucocytes. This may be seen from their contents, while several have been found actively surrounding small particles. Also in the larval gizzard region the muscle layer is reduced, and the liquefied muscular tissue is taken up by leucocytes which are most abundant in this region. However, the leucocytes do not seem to play an active part in the destruction of the muscles.

Histogenesis

Nearly all the methods of muscle regeneration so far described for the fore-gut may be assigned to the following types or modifications of these types:

1. The regeneration of the destroyed muscles from mesodermic cells, myocytes, which are found in the coelom near the inner sur-

face of the imaginal discs. This type of histogenesis is described for the fore-gut muscles of *Musca vomitoria* (Kowalevsky and Perez), *Gastrophilus* (Vaney), *Malacosoma castrensis* (Deegener '08), *Isosoma graminicola* (Van Leeuwen '07), and some others.

2. The regeneration of the imaginal muscles from the larval muscle nuclei and cytoplasm. The fibrillar part is destroyed while the nuclei persist, divide and form myoblasts. These form new fibrillae. This view, or a modification of it, is held by Korotneff ('92), Anglas ('00) for some muscles, Breed ('03), Deegener ('04) and Berlese.

3. The regeneration of the imaginal muscles from small imaginal nuclei present in the muscles. These nuclei become active at the time of pupation, while most or all of the larval nuclei degenerate. This type of regeneration is a complicated one and the investigators who advance this theory always admit that at a certain period it is impossible to tell whether one is dealing with rejuvenated larval nuclei or the so-called imaginal nuclei. This view has been put forward by Karawaiew ('98) for *Lasius niger* and Russ ('08) for *Anabolia laevis* (Trichoptera).

In discussing the histolysis of the fore-gut muscles of *Corydalis* I stated that this process ended about four days after the time of pupation. As the changes in the epithelium are not governed by the time element so much as by the individual pupal conditions so also is the muscle histolysis and histogenesis.

By examining figure 22 one notes that the larval nuclei clearly show a rejuvenated condition. They are arranging themselves in rows, while in the cross sections of the longitudinal muscles one can see faint indications of forming fibrillae. Leucocytes are quite numerous and are filled with densely staining granules. In a slightly more advanced condition (fig. 23) the longitudinal muscles clearly show the cut ends of forming fibrillae. The circular muscles do not show well in the figure. It is probable that figure 23 shows about the same stage of muscle regeneration as figure 22 although figure 23 is from a pupa nearly eight days old. However, further cephalad in the latter the fibrillae show clearly in the circular muscles. During this time one finds nuclei

undergoing division scattered throughout the muscles. However, the number that divide is very small.

Figure 24 shows a more advanced stage in muscle regeneration. The longitudinal muscles show not only the cut ends of fibrillae but have assumed their definitive arrangement, side by side immediately beneath the epithelium. The fibrillae show distinctly in the circular muscles. Figure 25 shows a somewhat older condition while figure 26 shows the beginnings of the cross striations in the muscle fibers. In a pupa ten days old the muscles have assumed their definitive form, though the cross striations do not show as clearly as they will become in the adult.

The type of histogenesis of the intestinal muscles in *Corydalis* is that described under (2). We have first a chemical liquefying of the muscles in place, beginning around the nuclei as centers. The leucocytes, which surround and penetrate between the muscle layers, undoubtedly take up and digest some of the broken down tissues. After the chemical liquefaction the larval nuclei, though some of them degenerate, become rejuvenated, and from them and the surrounding cytoplasm are built up the imaginal muscles.

CONCLUSIONS

1. The fore-gut of the larva of *Corydalis* may be divided into five well marked regions: pharynx, oesophagus, gizzard, portion between gizzard and oesophageal valve, and oesophageal valve.

2. The pharynx is provided with a series of dilator muscles attached to the walls of the head. The oesophagus presents no features of peculiar interest except perhaps the large number of longitudinal folds which permit a very great distension of its lumen.

3. The gizzard is well developed. Although Plateau ('74) and a number of workers following him have assigned to the gizzard only a straining function, it is evident that in *Corydalis* it exercises a grinding and crushing action as well. This is evidenced by the development and arrangement of muscles, the powerful chitinous teeth and their method of interlocking. Bordas ('05)

observed the rhythmic contraction and expansion of the gizzard in living Vespidae (*Vespa crabo*).

4. The gizzard does not pass directly into the oesophageal valve but is joined to it by a short, more or less smooth, tube.

5. The oesophageal valve is short and is lined with four strongly chitinized ridges which alternate with the caeca. At the point of union of the epithelium of mid- and fore-guts there is located a peculiar group of glandular cells whose function is doubtful.

6. The metamorphosis of the fore-gut is of a generalized type. The larval epithelium becomes partially broken down and the cells destroyed are replaced by the division of rejuvenated larval cells. The nuclei always divide mitotically and every spindle is located at the side of a vacuole. The dividing cell migrates towards the inner surface, though it retains a connection with the basement membrane.

7. The histolysis and histogenesis of the muscular coats are also generalized processes. The muscles liquefy in place. The greater number of the larval nuclei become rejuvenated and around them as centers the new fibrillar structures are developed.

8. The rôle of the leucocytes is a comparatively unimportant one. The leucocytes are present throughout the pupal life and are seen to engulf small particles of the broken down tissues. They do not take any active part in the destruction of the larval muscles or epithelium.

BIBLIOGRAPHY

- ANGLAS, J. 1898 Sur l'histolyse et l'histogenese du tube digestif des Hyménoptères pendant la métamorphose. C. R. Soc. Biol., pp. 1167-1170.
- 1899 Sur l'histolyse et l'histogenese des muscles des Hyménoptères pendant le métamorphose. C. R. Soc. Biol., pp. 931-933.
- 1900 a Note preliminaire sur la métamorphose internes de la guepe et de l'abeille. Le lyocytose. C. R. Soc. Biol., T. 52, pp. 94-97.
- 1900 b Sur la signifiacance des termes 'phagocytose' et 'lyocytose.' C. R. Soc. Biol., T. 52, pp. 219-221.
- 1901 a Observations sur les métamorphoses internes de la guepe et de l'abeille. Bull. Sci. France et Belgique, T. 34, pp. 363-473, plates 19-23.
- 1901 b Quelques caractères essentiels de l'histolyse pendant la métamorphose. Bull. Soc. Ent. France, pp. 301-304.
- 1902 a Le phénomènes des métamorphose internes. Scientia, Series Biol., T. 17, Paris, 84 pages.
- 1902 b Nouvelles observations sur les métamorphoses internes. Arch. d'Anat. Micr., T. 5, pp. 78-121.
- 1904 a De l'origin des cellules de remplacement de l'intestine chez les Hyménoptères. C. R. Soc. Biol., T. 56, pp. 173-174.
- 1904 b Du rôle des trachées dans la métamorphose des insectes. C. R. Soc. Biol., T. 56, pp. 175-176.
- BATAILLON, E. 1900 a Le problème des métamorphoses. C. R. Soc. Biol., T. 52, pp. 244-247.
- 1900 b Le théorie des métamorphoses de M. Ch. Perez. Bull. Soc. Ent. France, pp. 58-62.
- BERLESE, A. 1898 Osservazioni sopra particolari fenomeni che avvengono nella ninfosi des Muscidi. Riv. Patol. veg., T. 6, pp. 269-272.
- 1899 Osservazioni su fenomeni che avvengono la ninfosi degli insetti metabolici. Parte 1. Tissuto adiposi (Trofifitti). Riv. Patol. veg., T. 7, pp. 1-155.
- 1900 Considerazioni sulla fagotitosis negli insetti metabolici. Zool. Anz., Bd. 23, pp. 441-449.
- 1901 Vorgänge welche während der Metamorphose der metabolischen Insecten vorkommen. Zool. Anz., Bd. 24, pp. 515-521.
- 1902 a Osservazioni su fenomeni che avvengono durante la ninfosi degli insetti metabolici. Parte 1^a. Tissuto adiposa. Memoria seconda. (Lepidotteri, Imenotteri, Neurotteri, Coleotteri). Riv. Patol. veg., T. 9., pp 177-344.
- 1902 b Parte II. Tissuto muscolare (Miociti). Riv. Patol. veg., T. 10, pp. 1-120.

- BOAS, J. E. W. 1899 Einige Bemerkungen über die Metamorphose der Insecten. Zool. Jahrb., Syst., Bd. 12, pp. 385-402.
- BREED, R. S. 1903 The changes which occur in the muscles of a beetle, *Thymalus marginicollis* Chevr., during metamorphosis. Bull. Mus. Comp. Zool., Harvard College, vol. 40, pp. 317-382.
- BRUNELLI, G. 1902 Sul significato della metamorfosi negli insetti. Riv. Ital. Sci. Nat., T. 22, pp. 100-106.
- BRUYNE, C. DE. 1898 Recherches au sujet l'intervention de la phagocytose dans le développement des invertébrés. Arch. de Biologie, T. 15, pp. 181-300.
- CUENOT, L. 1899 Les prétendus organes phagocytaires décrits par Koulvetch chez la Blatta. Arch. Zool. Exp., Notes, pp. 1-2.
- DEEGENER, P. 1900 Entwicklung der Mundwerkzeuge und des Darmkanals von *Hydrophilus*. Zeitschr. f. wiss. Zool., Bd. 68, pp. 113-168.
- 1903 Zur postembryonalen Entwicklung des Insectendarms. Zool. Anz., 26, pp. 547-550.
- 1904 Die Entwicklung des Darmkanals der Insecten während der Metamorphose. Zool. Jahrb. Anat., 20, pp. 499-676.
- 1908 Die Entwicklung des Darmkanals der Insecten während Metamorphose. II Theil. *Malacosoma castrensis*. Zool. Jahrb. Anat., Bd. 26, pp. 45-182.
- 1909 Die Metamorphose der Insecten. Berlin. 56 pages.
- DEWITZ, J. 1902 a Untersuchungen über die Verwandlung der Insektenlarven. Arch. Physiol. (Engelmann), pp. 327-340.
- 1902 b Weitere Mittheilungen zu meinen Untersuchungen über die Verwandlung der Insektenlarven. Arch. Physiol. (Engelmann), pp. 425-442.
- FARKAS, K. 1903 Beiträge zu Energetik der Ontogenese. Dritte Mittheilung. Ueber Energieumsatz des Seidenspinnens während der Entwicklung im Ei and während Metamorphose. Arch. ges. Physiol., Bd. 98, pp. 490-546.
- FRENZEL, J. 1885 Einiges über den Mitteldarm der Insekten über epithelial-regeneration. Arch. mikr. Anat., Bd. 26, pp. 229-306.
- GANIN, M. 1876 Zur postembryonalen Entwicklung der Musciden. Warschau. Abdruck aus den Arbeiten der 5 Versammlung russischer Naturf. und Ärzte im Warschau.
- HEYMONS, R. 1907 Die verschiedenen Formen der Insectenmetamorphose und ihre Bedeutung im vergleich zur Metamorphose andere Arthropoden. Ergeb. Zool., Jena, Bd. 1, pp. 137-188.
- JUSBASCHJANZ, S. 1910 Zur Kenntniss der nachembryonalen Entwicklung der Stratiomyden. Jenaische Zeitschr. f. Naturwiss., Bd. 46, pp. 681-736.

- LUBBEN, H. 1907 Die innere Metamorphose der Trichopteren. Zool. Jahrb. Abth. Anat., Bd. 24, pp. 71-128.
- KARAWAIEW, W. 1898 Die nachembryonalen Entwicklung von *Lasius flavus*. Zeitschr. f. wiss. Zool., Bd. 64, pp. 385-478.
- 1899 Ueber Anatomie und Metamorphose des Darmkanals der larve von *Anobium paniceum*. Biol. Centralblatt, Bd. 19, pp. 122-130; 161-171.
- KOWALEVSKY, A. 1885 Beiträge zur nachembryonalen Entwicklung der Musciden. Zool. Anz., Bd. 8, pp. 98-99.
- 1887 Beiträge zur Kenntniss der nachembryonalen Entwicklung der Musciden. I Theil. Zeit. f. wiss. Zool., Bd. 45, pp. 542-594.
- KUNCKEL D'HERCULAIS, J. 1875 Recherches sur l'organisation et le développement des Volucelles. Paris.
- MERCIER, M. 1906 Les processus phagocytaires pendant le métamorphose des Batraciens Anoures et des insectes. Arch. Zool. Exp. et Gen., T. 5 (4), pp. 1-151.
- METSCHNIKOFF, E. 1883 Untersuchungen über die intracelluläre Verdauung bei wirbellosen Thieren. Arb. Zool. Inst., Wien., 5 Bd.
- 1892 Atrophie des muscles pendant le transformation des Batraciens. Ann. Inst. Pasteur, Bd. 6.
- METALNIKOFF, S. 1907 Zur Verwandlung der Insecten. Biol. Centralblatt, Bd. 27, pp. 396-405.
- MOBUSCZ, A. 1897 Ueber den Darmkanal der *Anthrenus* Larve, nebst bemerkungen zur Epithelialregeneration. Archiv f. Naturgesch., Bd. 62, pp. 89-128.
- NASSONOW, N. 1886 Zur postembryonalen Entwicklung der Ameise, *Lasius flavus*. Vorläufige Mittheilung (russisch). Sitz. d. zool. Abth. d. Gesellsch. d. Freunde d. Naturw.
- NEEDHAM, J. G. 1900 Some general features of the metamorphoses of the flag weevil, *Mononychus vulpeculus* Fabr. Biol. Bull., vol. 1, pp. 179-191.
- PEREZ, CH. 1899 Sur la métamorphose des insectes. Bull. Soc. Ent. France, T. 68, pp. 398-402.
- 1900 Sur l'histolyse musculaire chez les insectes. C. R. Soc. Biol., T. 52, pp. 7-8.
- 1910 Métamorphose de Muscides (*Calliphora erythrocephala* Mg.) Arch. de Zool. Exp., T. 4 (5), pp. 1-274.
- RENGEL, C. 1896 Ueber die Veränderungen des Darmepithels bei *Tenebrio molitor* während Metamorphose. Zeitschr. f. wiss. Zool., Bd. 62, pp. 1-60.
- ROUGET, C. 1900 La phagocytose et les leucocytes hematophages. C. R. Soc. Biol., T. 52, pp. 307-310.

- RUSS, E. A. L. 1908 Die postembryonale Entwicklung des Darmcanals bei Trichopteren (Anabolia laevis Zett.) Zool. Jahrb., Abth. Anat., Bd. 25, pp. 673-770.
- SCHINDLER, K. 1903 Die Metamorphose der Insecten. Zeitschr. Naturw., Bd. 75, pp. 341-356.
- TERRE, L. 1900 a Contribution a l'étude de l'histolyse et de l'histogenese du tissu musculaire chez l'abeille. C. R. Soc. Biol., T. 52, pp. 91-93.
1900 b Métamorphose et phagocytose. C. R. Soc. Biol., T. 52, pp. 158-159.
- VAN LEEUWEN, W. 1907 Beiträge zur Kenntniss der Metamorphosen. Die microscopische Anatomie des Darmkanals und dessen Drüsen von Isosoma graminicola Giraud. Tijdschr. d. Ned. Dierk. Vereen (2).
- VANEY, C. 1900 Contribution a l'étude des phénomènes de Métamorphose chez les Diptères. C. R. Acad. Sci., T. 131, pp. 758-760.
1902 Contribution a l'étude des larves et des metamorphoses des Diptères. These de Lyon. Ann. de l'Univ. de Lyon (n.s.) T. 1, Fasc. 9, 178 pp., 4 plates.
- VAN REES, J. 1889 Beiträge zur Kenntniss der innere Metamorphose von Musca vomitoria. Zool. Jahrb., Abth. Anat. u. Ontog., Bd. 3, pp. 1-134.
- VERSION, E. 1898 a La evoluzione del tubo intestinale nel Filugello. Continuazione e fine. Atti. Ist. Veneto Sc., T. 56, pp. 1273-1315.
1898 b L'évolution du tube intestinal chez le ver a Soie. Arch. Ital. Biol., T. 30, pp. 360-362.
1905 Zur Entwicklung Verdauungscanals bei Bombyx mori. Zeitschr. f. wiss. Zool., Bd. 82, pp. 523-600.
- VIALLANES, H. 1882 Histologie et développement des Insectes. Ann. de Sci. Nat., Zool., T. (6), T. 14 (1), pp. 348.
- WEISMANN, A. 1864 Die nachembryonalen Entwicklung der Musciden. Zeitschr. f. wiss. Zool., Bd. 14, pp. 264-336.
1866 Die Metamorphose von Corethra plumicornis. Zeitschr. f. wiss. Zool., Bd. 16, pp. 45.

PLATE 1

EXPLANATION OF FIGURES

1 Ventral view of the intestine of *Corydalis*, showing tracheal connections; *1th*, first thoracic spiracle; *2th*, second thoracic spiracle; *1ab*, *2ab*, *3ab*, *4ab*, abdominal gills; *k*, gizzard; *n*, nerve. The remaining letters are fully explained in the text.

2 A more enlarged view of the tracheal supply of a portion of the intestine. Letters fully explained in text.

3 A view of the muscular system of the fore-gut, showing the wonderful net-like arrangement.

4 The gizzard spread open; *s.r.*, small ridges; *l.r.*, large ridges. The posterior end of the gizzard is at the upper part of the photograph.

5 One of the large ridges of the gizzard in lateral view. *a.t.*, anterior tooth; *p.t.*, posterior tooth.

6 One of the large ridges of the gizzard in side view. There are three of the ridges as shown in figure 5, three as in figure 6, and six small ridges as shown in figure 7 in each gizzard.

7 One of the small ridges of the gizzard.

8 Cross-section of the oesophagus, showing the numerous folds.

9 Cross-section of the fore-gut near the middle of the gizzard.

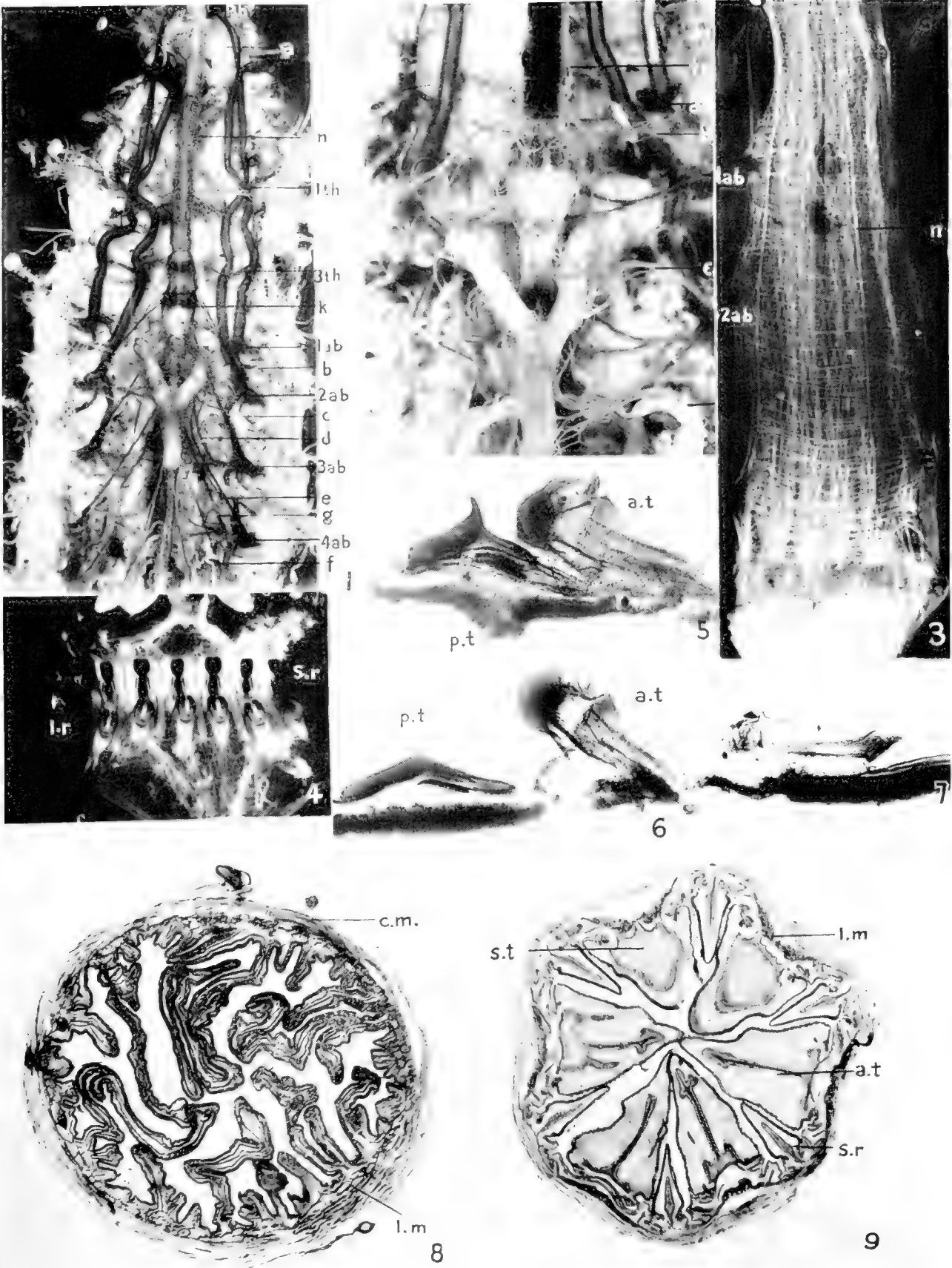


PLATE 2

EXPLANATION OF FIGURES

10 The point of union between the epithelium of the fore- and mid-guts. *ep.*, epithelium of the mid-gut; *g.e.*, glandular appearing epithelium of the fore-gut.

11 Fore- and mid-guts of the pupa just after pupation. In this case the intima of the gizzard remained lodged in the anterior end of the oesophagus.

12 Cross-section of the proventriculus of the fore-gut showing the four prominent chitinous ridges which project into the mid-gut.

13 Cross-section of the fore-gut of a pupa about twenty-four hours old *l.m.*, longitudinal muscles; *c.m.*, circular muscles; *fb.*, remnants of the breaking down muscle fibrillae; *x*, degenerating nuclei. Within the lumen lies the loosened intima.

14 A more highly magnified section of the epithelium of figure 13 to show the degenerating nuclei (*x*).

15 A lateral view of the muscles of the pharynx, showing the dorsal muscles in place.

16 Ventral view of the pharynx showing the lateral muscles.

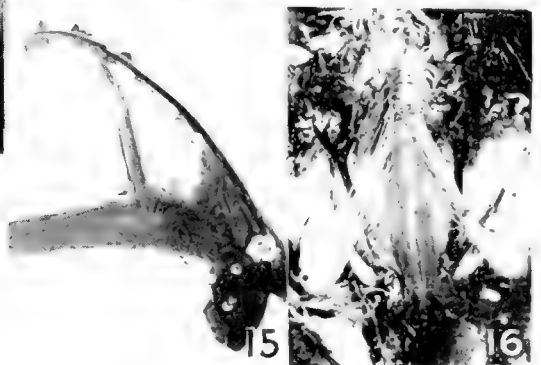
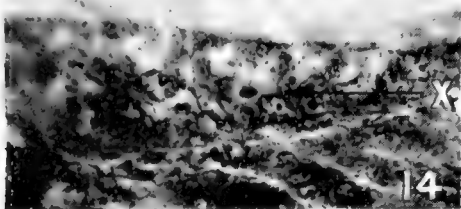
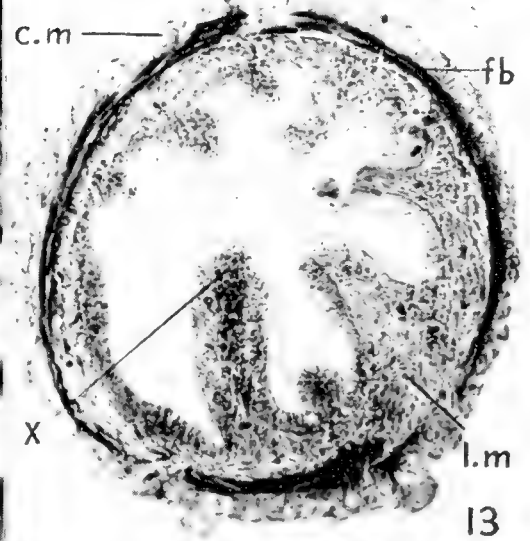
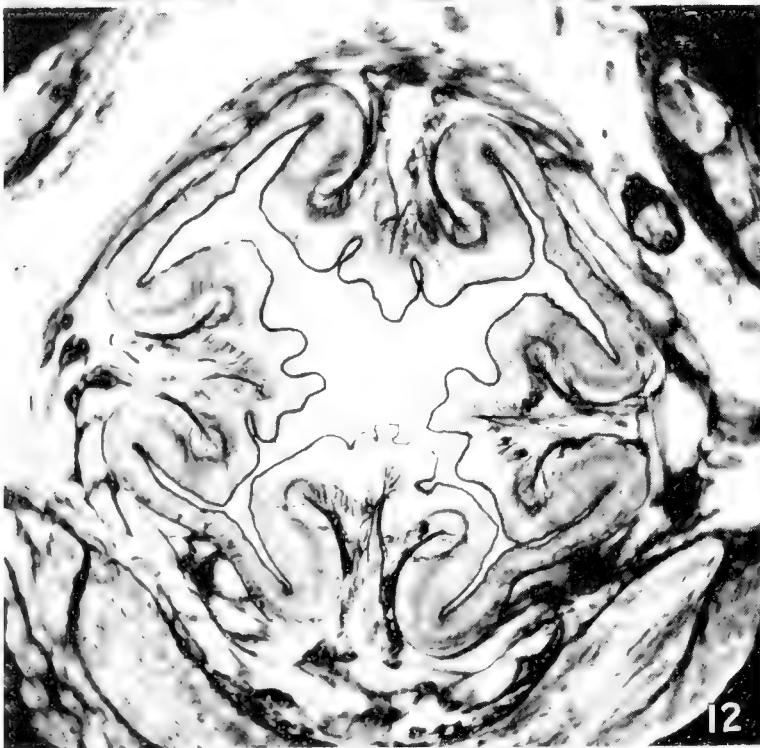
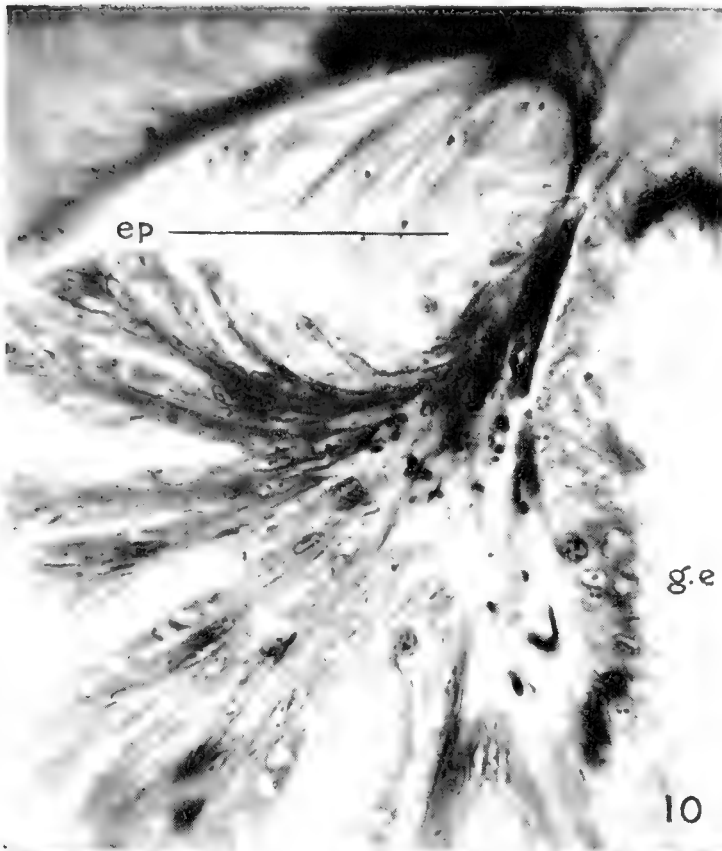


PLATE 3

EXPLANATION OF FIGURES

17 Cross-section of the wall of the larval fore-gut to show the structure of the epithelium and muscles. The following series of photographs are all taken at about the same point to show the series of changes in the epithelium and muscles during metamorphosis.

18 Cross-section of intestinal wall of a prepupa showing the earliest changes in the epithelium and muscles.

19 Cross-section from the same region as figure 18 from a prepupa in which the intima has been cast off.

20 Cross-section of the intestine of a young pupa. The nuclei of the muscles are now much more prominent and the pupal epithelium has secreted a new intima.

21 From a pupa about twenty-four hours old. Note the degeneration of the muscles, only a densely staining mass of fibrillae remaining. The epithelium has become much enlarged and vacuolate.

22 From a pupa ninety-six hours after pupation. The pupal intima has just been shed; *x*, degenerating nuclei. Note the embryonic appearance of the muscle nuclei. The histolysis of the muscles is now complete and we have the beginnings of histogenesis.

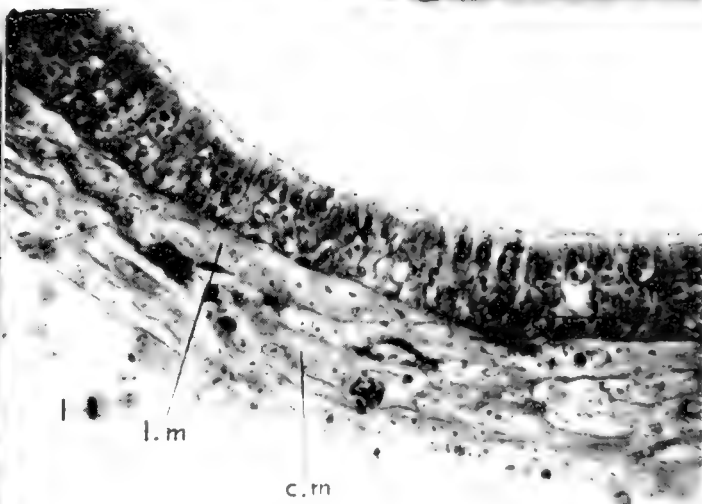
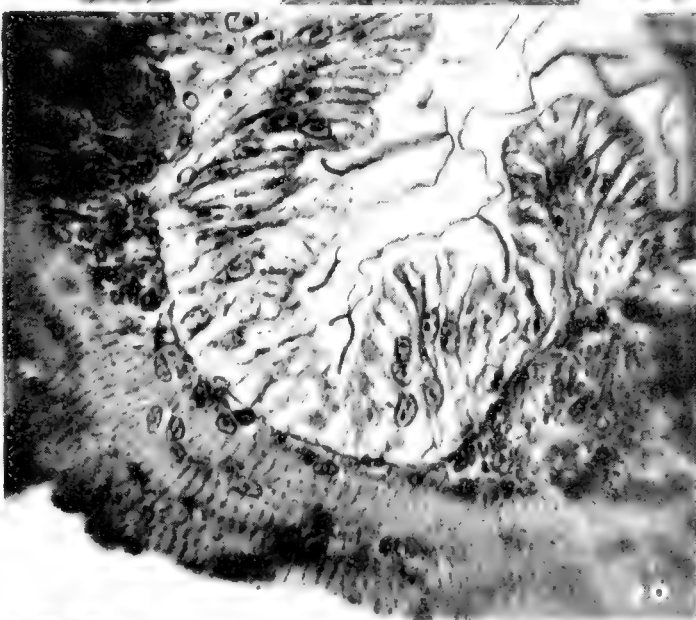


PLATE 4

EXPLANATION OF FIGURES

23 From a pupa slightly older than that of figure 22. Histogenesis has progressed somewhat further. Note the cut ends of fibrillae in the longitudinal muscles (*l.m.*).

24 A stage yet more advanced in the histogenesis of the imaginal muscles.

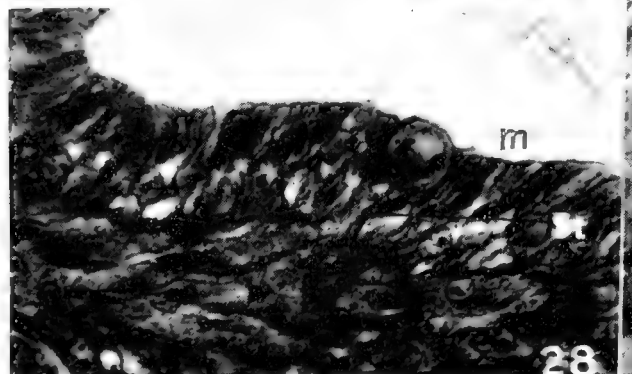
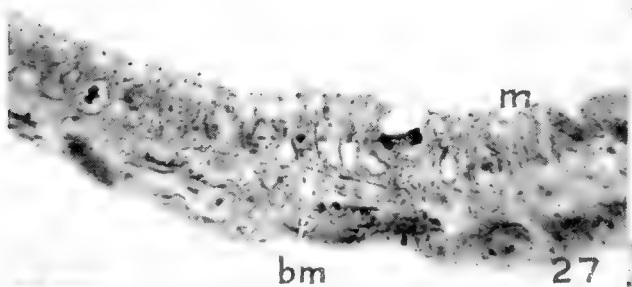
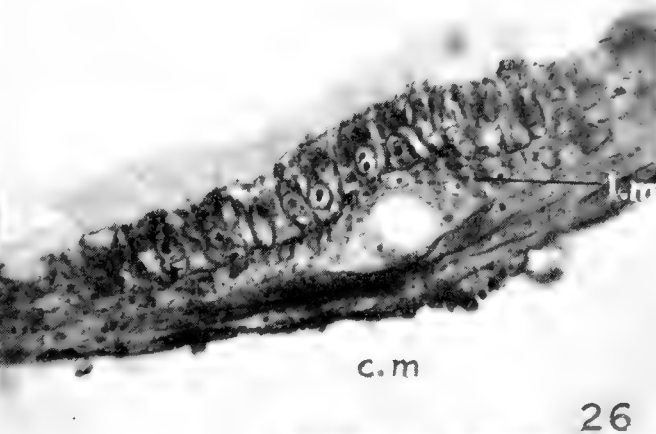
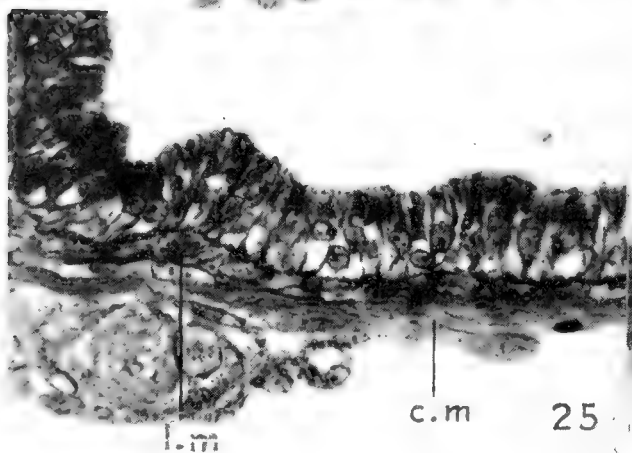
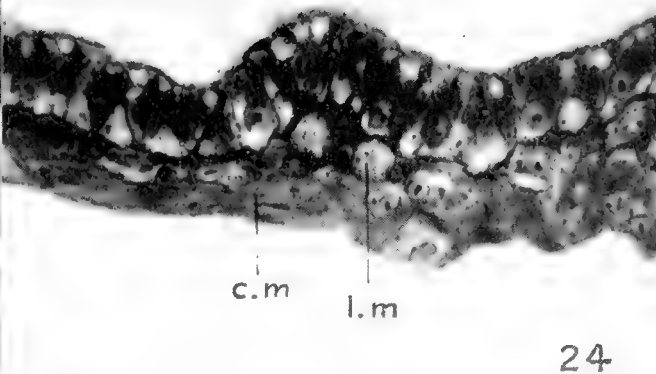
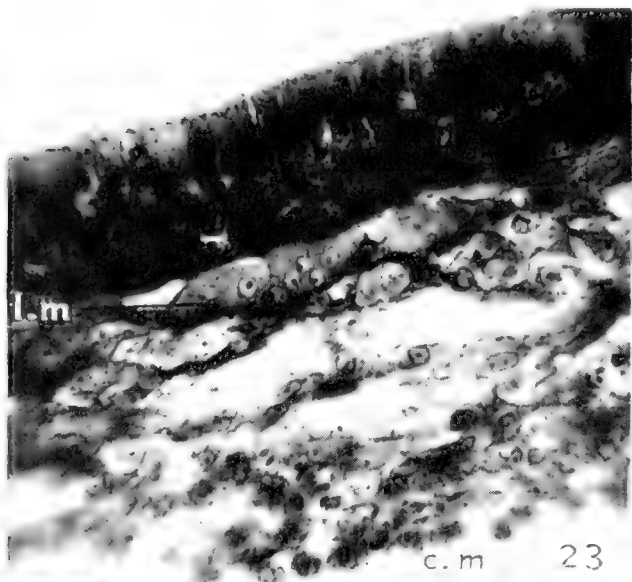
25 Note the forming fibrillae in the circular and longitudinal muscles. The muscles are now arranged in their two definite layers and the epithelium is now reformed. From an older pupa.

26 Cross striations can now be observed in the newly re-formed circular muscles (*c.m.*). These striations are faint and remain so till the emergence of the adult. *g.m.*, granular mass occupying the space between the loosened intima and the epithelium. From a pupa 160 hours old.

27 This figure shows the characteristic method of cell division; *m*, dividing cell; *st.*, strand connecting the cell with the basement membrane; *b.m.*, note the distinct basement membrane between the epithelium and the muscular layer.

28 Shows a condition similar to figure 27.

29 The epithelium from a pupa forty-eight hours old; *r*, row of black dots near the outer ends of the epithelial cells where the imaginal intima will be re-formed.



CHROMODORIS ZEBRA HEILPRIN: A DISTINCT SPECIES

W. M. SMALLWOOD AND ELIZABETH G. CLARK

Contributions from the Bermuda Biological Station for Research No. 26, and from the Zoological Laboratory of Syracuse University

SIX FIGURES

The following paper supplements one published by the senior author (Smallwood, '10). The two papers present the general morphology of *Chromodoris zebra* and point out the characters which distinguish it from *C. villafranca*. Bergh ('92) believed the two to be identical. The first paper gave a description of the external anatomy of *C. zebra*—the present one, the internal anatomy of the species. While our study of the internal anatomy has shown no marked deviation from the general plan of organization of the Doridae,—so well described by Eliot ('10, pp. 36 to 49) with *Doris tuberculata* as example,—it has furnished information concerning the anatomical details of this species not hitherto published.

DIGESTIVE SYSTEM

The mouth leads into a short but rather wide atrial chamber (6 mm. long by 9 mm. in diameter), the inner wall of which is lamellated. At the posterior end of this chamber a ring-shaped depression forms the boundary between the lateral wall and the posterior wall, the latter constituting the labial disc. A vertical slit-like opening in this disc is the entrance to a passage extending posteriorly for 4 mm.; this passage is lined with closely set spines, which form what is sometimes incorrectly termed a 'prehensile collar.' This spinous passage expands at its posterior end into the main buccal cavity, 5 mm. in length by 6 mm. in diameter. The floor of the main buccal cavity is formed by the odontophore, a round muscular prominence bearing the radula and divided into

lateral halves by a longitudinal furrow. The walls of the buccal cavity are chiefly composed of muscles and are greatly thickened ventrally. The entire mass is cylindrical (fig. 2, *phx.*).

The radula, borne upon the rounded prominence forming the floor of the buccal cavity, is folded in the middle and fits into the longitudinal groove; it emerges from the curved sac which lies at the ventro-posterior angle of the buccal mass. This sac is lined with cells whose special function is the secretion of new teeth. The total number of rows of teeth is 95 to 100; of these 20 to 30 are in the radula sac, the posterior 4 to 5 being in process of formation. The median tooth is absent. In each transverse row are 165 to 170 teeth on each side. All of the pleural teeth bear two

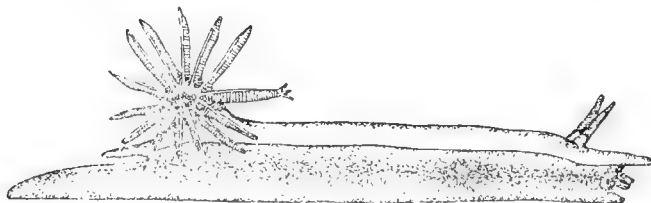


Fig. 1 *Chromodoris zebra* Heilprin. Viewed from the right side; the branchial-rosette turned toward the observer; $\frac{3}{8}$ natural size. Copied from Smallwood, '10.

denticles; one long denticle terminating in a sharp slender point, and a shorter one with a slightly blunter distal end (fig. 3). Slight variations in size and form of teeth occur, as may be seen in figure 3, and as they near the margin, we find that the denticles gradually become more and more blunt, until at the outermost edge, they are mere rounded knobs supported by the broader base. In some teeth, the denticles are entirely lacking, doubtless having been worn off. The posterior margin of the tooth, that is, the side toward which the denticles point, is slightly crenulated. All teeth are of nearly the same size, measuring 20 micra long, 9 micra wide, and 3 micra thick. The longest denticle measured was 6 micra long.

Bergh ('78, pp. 23, 29 and 30) gives the following for *Chromodoris villafranca*:

Forma corporis gracilis. Pedamentum foliorum branchialium humile. Color paginae superioris glaucescens, fasciis transversalibus latioribus caeruleis dilutis et praesertim lineis fulvis (ut plurimum 7) non semper

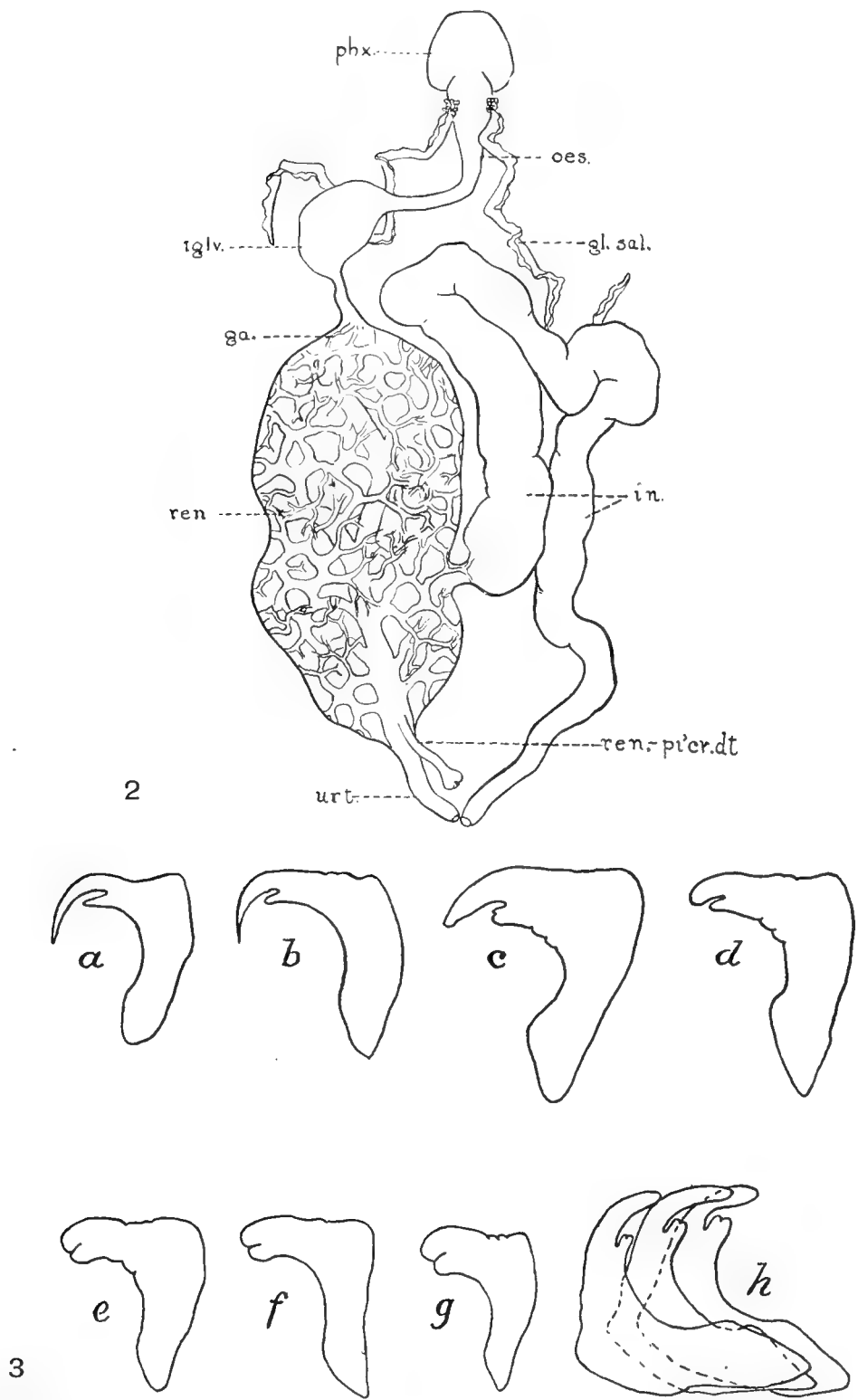


Fig. 2 Digestive and renal systems with the intestine displaced; *ga.*, stomach; *gl.sal.*, salivary gland; *iglv.*, crop; *in.*, intestine; *oes.*, oesophagus; *phx.*, buccal mass; *ren.*, kidney; *ren.-pi'cr.dt.*, reno-pericardial duct; *urt.*, ureter.

Fig. 3 Pleural teeth, showing the successive steps in loss of denticles from *a* to *g*, also the arrangement of the teeth in relation to each other.

inter se distinctis et saepe divisis ornatus; margo dorsalis fulvus; clavus rhinophoriorum caeruleus, margine posteriore linea fulva ornatus; folia branchialia rhachide extus linea punctorum fulvorum ornata; latera corporis caerulescentia lineis fulvis 3-4 longitudinalibus (ut in dorso) ornata; podarium infra caerulescens vel glaucescens.

Dentes radulae grosse denticulati.

Hab. M. mediterr. (M. adriat., Napoli, Panormi, Genova, Bona).

Die Mundröhre etwa 4 mm. lang, von schön grün-blauer Farbe, ganz wie bei der vorigen Art [*C. elegans*]. Der Schlundkopf etwa 6 mm. lang bei einer Breite von 3 und einer Höhe bis 3 mm.; die Raspelscheide hinten und unten am Schlundkopfe etwa 1 mm. hervortretend, mit der schmutzig gras-grünen Rassel stark hindurchschimmernd. Die Lippenplatten grau-gelb, sonst ganz wie bei der vorigen Art; die Elemente derselben ganz wie bei jener, vielleicht etwas deutlicher am Grunde des Hakens gestreift. Die Zunge wie oben; die Rassel zeigte 26 Zahnplattenreihen, von denen die ersten 5 incomplet; weiter gegen hinten fanden sich noch 32 entwickelte und 5 junge Reihen, die Gesamtzahl derselben somit 63 betragend. In den hintersten Reihen der Zunge fanden sich (jederseits) 85 Platten, und die Anzahl stieg weiter gegen hinten bis 90. Die Platten von schwach-gelblicher Farbe, denen der vorigen Art im Ganzen ziemlich ähnlich; die Höhe der äussersten meistens etwa 0,04-0,07 mm. betragend, die der Platten sich im Ganzen bis 0,14 mm. erhebend. Die äussersten (Fig. 9a) Platten niedriger und mit stärkerer Denticulation. Die Platten im Ganzen stärker und alle am Aussenrande und zwar stark denticuliert (Fig. 5-9), die innerste Platte mit einem starken Dentikel innen am Grunde des Hakens (Fig. 5aa.).

A comparison of these two descriptions shows the following differences: The rows of teeth in *C. zebra* are 95 to 100, in *C. villafranca* 63. The number of teeth in a row in *C. zebra* is 165 to 170, while in *C. villafranca* it is but 85 to 90. The form of the teeth as shown in Bergh's figures indicates clearly that the two forms belong to distinct species.

The oesophagus

The oesophagus of *C. zebra* leaves the pharynx at the dorso-posterior angle of the buccal mass. On each side, in a slightly dorsal position, the ducts from the single pair of salivary glands (fig. 2, *gl.sal.*) enter the oesophagus. These glands are long ribbon-like structures, folded along their mid-line and somewhat irregular on the margins. They are 3 cm. long and 2 mm. wide. From this point the oesophagus (*oes.*) continues posteriorly as

a slender tube for 1 cm., passes through the nervous collar, and then expands into a thin-walled sac (15 mm. long by 5 mm. in diameter), the crop (*iglv.*). From the crop it continues, as a slightly broader tube (3 mm. in diameter), to the anterior portion of the liver mass, where it passes directly into the anterior end of the stomach (fig. 2, *ga.*). Throughout its entire length, the thin walls of the oesophagus bear longitudinal lamellae on their inner surface.

The stomach

The stomach is a large thin-walled sac, 30 mm. long by 15 mm. in diameter, lying within the anterior two-thirds of the liver mass. Its walls are slightly lamellated and perforated by innumerable large openings, the orifices of the evaginations which collectively constitute the 'liver' or digestive gland, so that apparently the cavity which we call the stomach is merely enclosed in a loose meshwork. Closely applied to the walls of the stomach are the ramifications of the nephridial organ (fig. 2, *ren.*).

The intestine

The intestine (fig. 2, *in.*) arises from the right side of the stomach about two-thirds the length of the stomach from its anterior end. This tube, at first rather narrow, has its greatest diameter (7 mm.) near its emergence from the stomach, and it gradually grows smaller as it nears the anal opening. From the stomach, the intestine passes anteriorly for about 25 mm. to the left side of the anterior end of the liver mass, where it turns abruptly to the right and runs diagonally crosswise and backwards for 10 mm.; then it bends again, continuing posteriorly for 40 mm., until it reaches the anal opening within the branchial circle. The walls bear longitudinal lamellae upon the interior, two especially conspicuous folds lying side by side upon the median ventral surface.

The liver

The liver occupies the posterior portion of the body cavity. It is a large pyriform organ, the broad anterior end being slightly bilobed in front. It is surrounded almost completely by the hermaphroditic gland, whose ramifications may be seen lying on the surface of the dark colored liver. In this species, the liver completely envelopes the stomach and the larger part of the kidney.

RENAL SYSTEM

It is impossible to determine anything very definite about the extent of the nephridial organ without careful histological study. However, it is possible to say that it consists of a main trunk with numerous lateral anastomosing ramifications, which cover the greater part of the dorsal surface of the stomach and probably run into the liver. A short ureter (fig. 2, *urt.*) leads from the kidney to the excretory orifice, a minute opening within the branchial circle close to the anal papilla. With the main trunk, where it merges into the ureter, is joined another duct (*ren.-pi'cr.dt.*), which arises from the pericardium, where a valvular enlargement allows the passage of fluid from the pericardium to the renal trunk.

RESPIRATORY AND CIRCULATORY SYSTEMS

These two closely related systems embrace the branchiae, the heart, the blood vessels and blood spaces, together with the blood gland. The heart lies within the pericardium on the upper surface of the posterior portion of the liver mass just anterior to the branchiae. It consists of an anterior muscular ventricle (fig. 4, *v.*) and a posterior thin-walled auricle (*aur.l.*). From the apex of the ventricle a large blood vessel runs forward, giving off lateral branches to the liver and other organs. Just anterior to the liver mass occurs an enlargement, which sends one vessel (*va.sng.gen.*) to the genitalia, one to the blood gland (*gl.sng.*) and another (*va.sng.buc.*) which continues anteriorly to surround the buccal mass. Throughout the integument are numerous blood spaces, which lead to the posterior end of the body and pour

their contents into the lateral blood vessels which enter the auricle at its posterior angles. It is probable that, as occurs in other forms, the entire primary body cavity surrounding the various organs is filled with the blood fluid and that this is collected and aerated in the branchiae, from which it passes directly into the middle portion of the auricle through the posterior median blood vessel of the auricle.

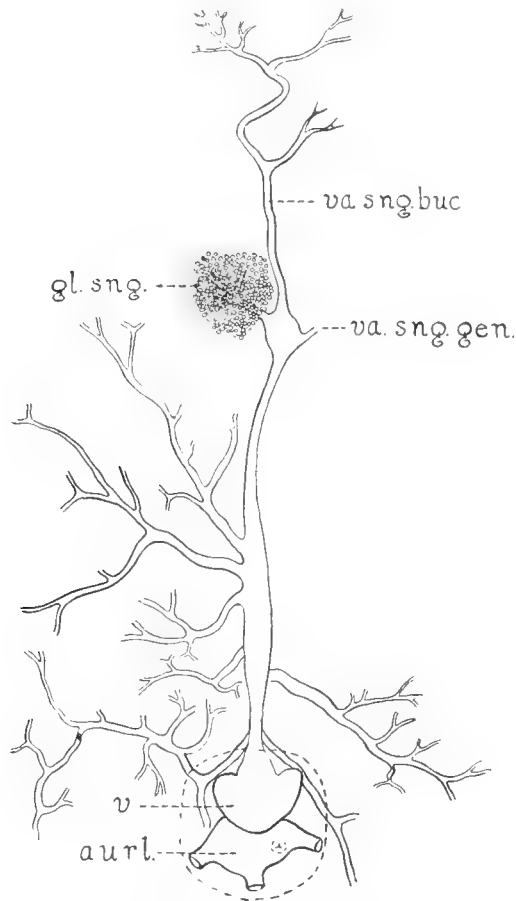


Fig. 4 Heart, blood gland and some of the more important blood vessels. *aurl.*, auricle; *gl.sng.*, blood gland; *v.*, ventricle; *va.sng.buc.*, blood vessel to buccal mass; *va.sng.gen.*, blood vessel to genitalia.

The blood gland (fig. 4, *gl.sng.*) is an irregular leaf-like mass made up of very small round cells. It lies immediately above the central nervous system and has an antero-posterior diameter of 7 mm. and a lateral diameter of 5 mm.

There are from 12 to 14 branchiae in the branchial circle. Each branchia is provided with an afferent and an efferent blood vessel, which carry the blood respectively to and from the branchiae.

NERVOUS SYSTEM

The nervous system consists of two main divisions—the central and the visceral or sympathetic part. The central part of the nervous system, enclosed within a semi-transparent membranous capsule, forms a nerve collar around the oesophagus, lying just beneath the blood gland. It is made up of three pairs of large ganglia,—two cerebral, two pleural and two pedal,—a small pair of buccal ganglia, and several minor ones.

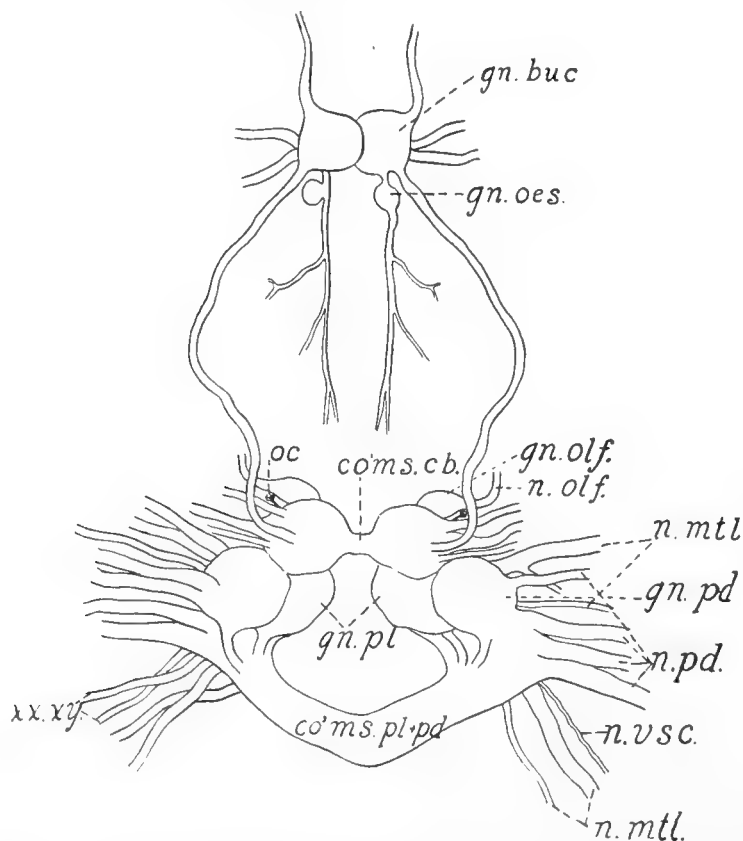


Fig. 5 Central nervous system seen from above and posteriorly. *co'ms.cb.*, cerebral commissure; *co'ms.pl.+pd.*, fused pleural and pedal commissures; *gn.buc.*, buccal ganglion; *gn.oes.*, oesophageal ganglion; *gn.olf.*, olfactory ganglion; *gn.pd.*, pedal ganglion; *gn.pl.*, pleural ganglia; *n.mtl.*, nerves to mantle; *n.olf.*, olfactory nerve; *n.pd.*, pedal nerves; *n.vsc.*, nerve to sympathetic system; *oc.*, eye; *xx, xy*, nerves to liver and genitalia.

The cerebral ganglia are the most anterior pair and are united with each other by a short (0.5 mm.) commissure (*co'ms.cb.*), as seen in figure 5. From the anterior margin of each projects the almost sessile olfactory ganglion (*gn.olf.*), which gradually

tapers off into the large olfactory nerve, that passes into the base of the rhinophore. Upon the dorsal surface, near the external margin of the cerebral ganglia, arise the two small optic ganglia, each of which sends a short nerve to the corresponding eye (*oc.*). Each cerebral ganglion gives rise to three pairs of nerves, which pass forward and innervate respectively the oral tentacles, the mouth, and the lips. Besides these nerves, each ganglion sends from its mid-ventral surface a connective which passes around the oesophagus to the buccal ganglia (*gn.buc.*); these lie, side by side, ventral to the oesophagus just behind the buccal mass. A slender nerve leads forward from each buccal ganglion to the tongue. Two lateral nerves from each buccal ganglion innervate the buccal mass. Posteriorly, a small ganglion (*gn.oes.*) is joined to each buccal ganglion by an exceedingly short nerve. This oesophageal ganglion sends down the oesophagus a single nerve, which has three branches that spread over the tube.

Lying more dorsal and slightly posterior to the cerebral ganglia, to which they are joined by very short connectives, are the equally large pleural ganglia (*gn.pl.*). These generally give rise to three pairs of nerves which innervate the mantle. From one of them arises on the right side a slender nerve (*n.vsc.*), which communicates with the visceral part of the nervous system.

The pedal ganglia (fig. 5, *gn.pd.*), not so closely united to the others, lie in a more lateral position. From 4 to 5 pairs of nerves arise from them. Of these, two pairs of the smaller nerves innervate the body wall, while of the remaining larger ones at least two pairs pass to the foot as anterior and posterior pedal nerves.

The pedal and pleural commissures (*co'ms.pl. + pd.*) unite into a single broad band, which encircles the oesophagus ventrally, the nerve fibers arising in each case from the posterior margin of the ganglion. Two large nerves (*xx.xy.*), arising close to this commissure, innervate the liver and genitalia. Their precise origin is difficult to ascertain. The remaining pairs of nerves are distributed posteriorly to the mantle.

The study of the central nervous system of this nudibranch brought out the fact that there is considerable variation in the total

number of nerves, as well as the number that arise from each ganglion. The apparent origin of the nerves as well as the relation of the ganglia to each other is variable. In some of the specimens studied the three pairs of ganglia comprising the nerve collar were so closely crowded together that it was well nigh impossible to discover their true relations.

The visceral or sympathetic part of the nervous system, as stated above, communicates with the cerebral nervous mass by means of a nerve (*n.vsc.*) from the right pleural ganglion. This, the visceral portion of the nervous system, consists of an irregular system of exceedingly fine nerves spreading entirely over the viscera and containing numerous small ganglia distributed along their course.

REPRODUCTIVE SYSTEM

The reproductive system is made up of two main portions; one, known as the anterior genital mass, is an irregularly rounded body made up of a complex of tubules, cavities and ducts, lying at the right anterior end of the body cavity; the other, the posterior genital mass, is the hermaphroditic gland, which spreads over the liver and is joined to the anterior genital mass by the hermaphroditic duct. The size and shape of both of these portions varies greatly at different seasons of the year, being enormously enlarged during the breeding season, the time at which this material was collected.

The posterior genital mass

The hermaphroditic gland spreads over the entire surface of the liver, from which it may be distinguished by its lighter color. It is made up of numerous lobules opening into a series of minute tubules, which finally emerge into two large ducts; these unite to form the hermaphroditic duct. In this gland both eggs and sperm have their origin. As the hermaphroditic duct (fig. 6, *dt.her.*) leaves the gland, it passes to the anterior genital mass, which it enters on the median side. It immediately enlarges into a thickened tube, the ampulla (*amp.*), which is 2 mm. wide

and about 5 mm. long. A little beyond the ampullar enlargement the duct bifurcates, giving rise to the vas deferens (*va.df.*) and the oviduct (*o'dt.*).

The anterior genital mass

The anterior genital mass is made up of male and female genitalia. The male genital apparatus is fairly simple. It consists of a coiled tube, the vas deferens, which extends from the bifurcation of the hermaphroditic duct to the enlargement known as the penis sheath (*ivlr.pe.*). From the point of bifurcation it

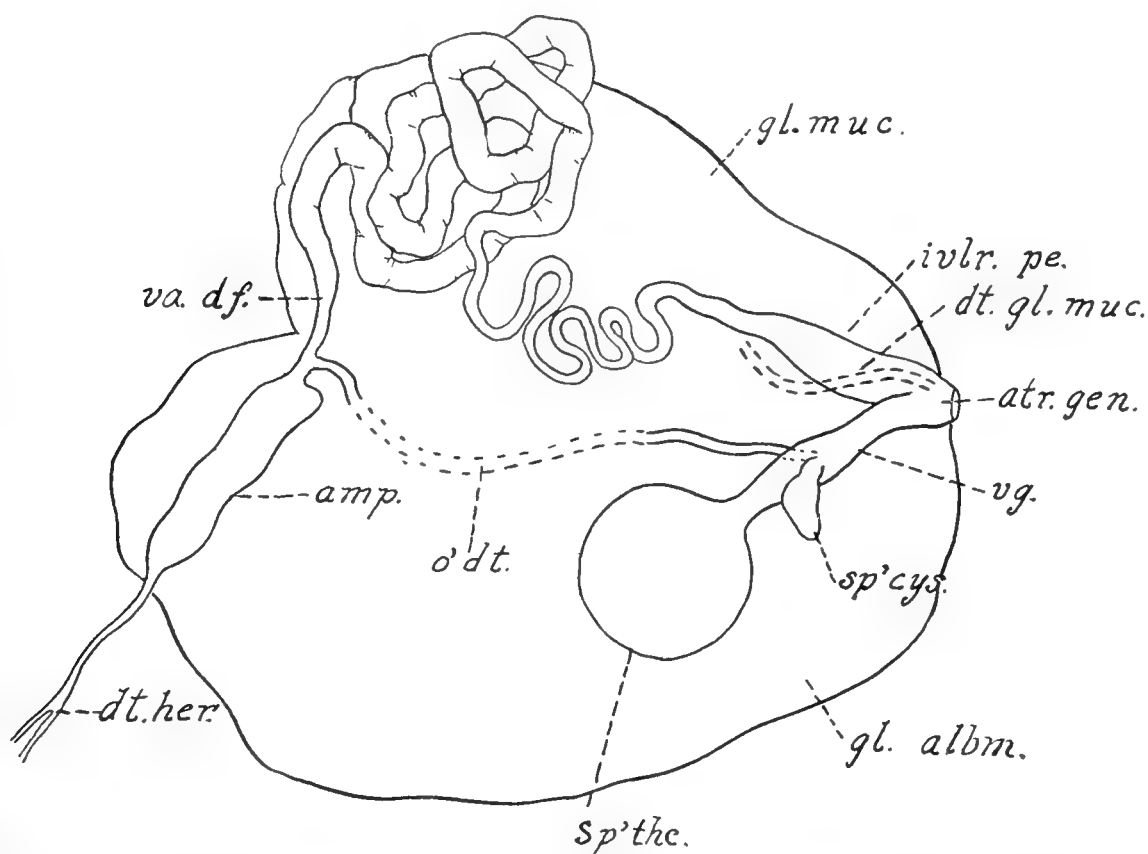


Fig. 6 Diagram of the reproductive glands and ducts. *amp.*, ampulla; *atr.gen.*, genital atrium; *dt.gl.muc.*, duct of mucous gland; *dt.her.*, duct of hermaphroditic gland; *gl.albm.*, albumen gland; *gl.muc.*, mucous gland; *o'dt.*, oviduct; *ivlr.pe.*, penis sheath; *sp'cys.*, spermatocyst; *sp'thc.*, spermatheca; *va.df.*, vas deferens; *vg.*, vagina.

gradually enlarges, the enlarged portion being thrown into numerous folds, which lie on the anterior mucous-gland portion of the mass. The terminal portion, perhaps a third of the length of the whole, is again narrowed and is very sinuous; it only gradually

enlarges to become continuous with the sheath of the penis, which, in turn, communicates with the genital atrium (*atr.gen.*).

The female genitalia are more complex. They may be roughly divided into two parts; the uterine or oviducal portion, and the portion specialized for the reception and storage of sperm, which is in direct communication with the oviduct. The oviduct leads from the bifurcation of the hermaphroditic duct directly to the albumen gland (*gl.albm.*), a large mass of closely convoluted tubules constituting the posterior portion of the anterior genital mass. From the albumen gland the duct passes forward into the mucous gland (*gl.muc.*), and terminates in the vagina (*vg.*), which is a single broad tube leading to the atrium. The mucous gland has a distinct duct (*dt.gl.muc.*) leading to the genital atrium. At its deep end the vagina expands into a rather large globular sac, which seems to be filled with detritus and a few free spermatozoa; this is the 'spermatotheca' of Bergh. About halfway between the external opening of the vagina and the spermatotheca a much smaller pear-shaped sac (*sp.cys.*) with thicker walls is connected with the vagina by a simple duct. This sac is the spermatocyst. It contains large numbers of spermatozoa. Just beneath this spermatocyst, on the ventral side of the vaginal tube, is the orifice through which the oviduct communicates with the vaginal portion of the female genitalia.

BIBLIOGRAPHY

- BERGH, R. 1878 Untersuchung der Chromodoris elegans und villafranca. Malakozool. Blätter für 1878, pp. 1-36, Taf. 1 und 2.
1892 System der nudibranchiaten Gasteropoden. In Karl Semper's Reisen im Archipel der Philippinen, Theil 2, Bd. 2, Heft 13, pp. 993-1165. Also separately, Wiesbaden: Kreidel, 173 pp.
- ELIOT, C. 1910 A monograph of the British Nudibranchiate Mollusca, Part viii (Supplementary). London, Ray Society, 198 pp., 8 pls.
- HEILPRIN, A. 1889 The Bermuda Islands. Philadelphia [vi] + 231 pp., 17 pls.
- SMALLWOOD, W. M. 1910 Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zool. Soc., London, Pt. I, pp. 137-145. (Contrib. Bermuda Biol. Sta. No. 18.)

PRIMITIVE REPTILES

A REVIEW

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ONE FIGURE

The evidence seems now conclusive that the extensive American reptilian fauna hitherto called Permian is in part of upper Pennsylvanian, in part of lower Permian age, and, therefore, is the oldest known. From other parts of the world there are only a few known forms supposed to be of equivalent, or approximately equivalent, age, the chief of which, if not the only ones, are *Stereosternum* and *Mesosaurus* from the Santa Catherina System of Brazil, the latter genus also from the upper Dwyka of South Africa. From immediately superjacent beds in South Africa, doubtless of lower Permian age, but one or two reptiles are known, *Archaeosuchus* and *Eccasaurus*, referred by Broom, the former at least, to the dinocephalian group of the Therapsida. From the Beaufort beds, upper and lower, of Africa, numerous genera of reptiles are known, referred to the Cotylosauria and Therapsida. Because of the close affinity or identity of some of these genera with those found in Russia, they doubtless should all be considered of upper Permian age, as distinguished from lower Permian. From the Rothliegende of Germany and France, of lower Permian age, the following genera of reptiles are known: *Stephanospondylus*, *Phanerosaurus*, *Datheosaurus*, *Stereorhachis*, *Kadaliosaurus*, *Callibrachion*, *Aphelosaurus*, and *Paleohatteria* (*Haptodus*). Leaving *Archaeosuchus* and *Eccasaurus* out of account in the following discussion, the present paper will deal with the carboniferous and lower Permian reptiles only, including

the genera mentioned above and the numerous ones known from the American Permian-carboniferous. Two other, disputed groups of contemporary air-breathers, believed to be reptiles by some authors of repute, will be discussed in the sequel.

All of the so-called orders represented by these reptiles are believed to be continuous, not only throughout the Permian of Europe or Africa, but also throughout more or less of the trias if not the mesozoic, though in most cases the later forms are either distinctly modified, or have reached a higher degree of specialization; and their discussion may be omitted here. *Pareiasaurus* and *Propappus*, for instance, from the upper Permian of Russia and Africa, have dorsal osseous scutes, a distinct acromial process of the scapula, and coössified calcaneum and astragalus, all unknown in the American *Cotylosauria*; characters, which in themselves are sufficient to separate the *Pareiasauria* as a distinct group. *Procolophon*, also, with its allies *Telerpeton*, *Sclerosaurus* and *Koiloskiosaurus* from the trias, have departed so far from the primitive simplicity of the earlier types of America in the skull structure and in the pectoral girdle as stated by Huene, that they may well be separated into a group by themselves. *Procolophon* has been supposed for years, to be a primitive form of the double-arched or 'Diapsidan' type, notwithstanding its occurrence in much later deposits than those of *Paleohatteria*. Huene has recently expressed the opinion, one I have had for the past five years, that the *Procolophonia* have nothing to do genetically with the rhynchocephalian or archæosaurian phylum.

Using then the term Permian-carboniferous to include the upper carboniferous and lower Permian, and omitting the imperfectly known *Archæosuchus* and *Eccasaurus*, we have perhaps thirty or more genera of undisputed reptiles which it will be of interest to compare directly.

The first and lowest group of these, the *Cotylosauria* as usually accepted, began in the upper Pennsylvanian of North America and continued into the *Procolophonia* of the trias of South Africa and Europe; it has generally been believed to be the order from which all later Amniota have been derived. The second order,

the Theromorpha of Cope as limited by me,¹ in part the Pelycosauria of authors, began also in carboniferous times and extended as an order into triassic times in Europe. The third group, the Proterosauria, as represented by *Paleohatteria*, *Proterosaurus* and less well known allied forms, began, so far as we know, in the middle Rothliegende of Germany and continued throughout the Permian; it is generally supposed to be the ancestral group from which all later true reptiles, that is the double-arched forms, arose, and is often included among the Rhynchocephalia, sens. lat. The fourth group, the Proganosauria, as originally defined by Baur and later restricted by Osborn and McGregor, has been accepted by some, though not by all students as an early group of the Sauropterygia, which continued to near the close of mesozoic times.

From a study of all these I have endeavored to summarize and correlate those structural characters upon which phylogenies and classification must depend. Many of the known forms are as yet represented by fragmentary and incomplete specimens only, whether those of America or of other lands; of the latter I have little or no autoptic knowledge, a fact less to be regretted since most of them, and those the most important ones, have been described and illustrated by competent students. Of the American forms I have studied with more or less care nearly all the known material, with the exception of that preserved in the museum at Munich, which has been ably discussed by Broili. Of this material I am familiar with complete or nearly complete skeletons pertaining to the following genera: *Diadectes* Cope, *Diasparactus* Case, *Limnoscelis* Will., *Seymouria* Broili, *Captorhinus* Cope, *Labidosaurus* Cope, *Varanosaurus* Broili, *Casea* Will., *Ophiacodon* Marsh and *Dimetrodon* Cope. I have also studied the less complete specimens belonging in the following genera: *Diadectoides* Case, *Pantylus* Cope, *Edaphosaurus* (*Naosaurus*) Cope,² *Sphenacodon* Marsh, *Clepsyrops* Cope, *Araeoscelis* Williston and *Animasaurus* C. and W., together with the more fragmentary material of nearly all the other known gen-

¹ American Permian Vertebrates, p. 70.

² The identity of *Naosaurus* with *Edaphosaurus* is now established.

era from Illinois, Kansas, Oklahoma, Texas, New Mexico and Colorado. And I need not add that I have diligently studied all the literature bearing on these American reptiles, especially that of Case and Broili. I am indebted to Dr. Case for the kindly criticism of the manuscript of the present article.

It will be understood that I give the characters so far as they seem to be established by numerous forms, though it is quite possible that future discoveries of new forms, or of better material of forms now imperfectly known, may require the removal of some of the characters included under the constant list to the inconstant or variable list. But the converse will not be possible unless I have committed errors, since here the evidence is positive, not negative. Among so many and diverse forms it must be evident that most of the characters common to all, so far as the material goes, are to be considered as primitive ones, that is, characters possessed by the earlier or earliest reptiles far back in Pennsylvania times. But, by no means are these the only primitive characters, since it is very evident that even by the close of the carboniferous not a few had become variable.

CONSTANT CHARACTERS OF PERMOCARBONIFEROUS REPTILES

Crawling reptiles from about 0.3 to about $2\frac{1}{2}$ meters in length. A parietal foramen, postorbital, postfrontal, lacrimal, nasal and probably at least one additional membranous cranial bone not occurring in *Sphenodon* present in the skull. Quadratojugal, paroccipital, epipterygoid and septomaxillary probably always distinct; parietals, frontals and nasals never fused in middle line; stapes probably always large; a separate prearticular³ bone; splenial entering symphysis; coronoid small; teeth on paired premaxillae, maxillae, palatines, pterygoids and dentaries; pterygoids articulating with vomers (prevomers), basisphenoid, palatines and quadrates; quadrate always fixed, though not always firmly attached.

³ This term (1903) had become established in paleontological literature before Gaupp ('08), in ignorance of its use, proposed the name goniale for the same bone.

Vertebrae notochordal (or at least deeply and conically biconcave); all vertebrae to the second-sixth caudal with intercentra and all to the eighth or ninth with ribs, those of the lumbar, sacral and basal caudal united more or less suturally with body and arch, but in all cases the capitulum more or less intercentral in position. Capitulum of free ribs articulating intercentrally, resting more or less on the front end of the centrum, but not articulating with a parapophysial process. Chevrons present on all the caudals, save the first two-six and the extreme terminal ones. Proatlas probably always present. Atlas composed of a large free odontoid supporting the paired neural arch chiefly, and an intercentrum not much larger than the one following

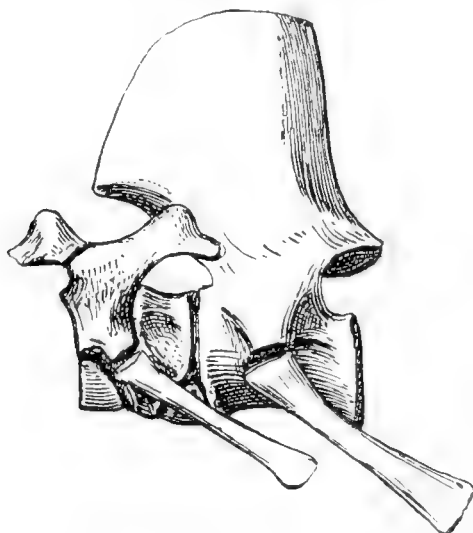


Fig. 1 Proatlas, atlas and axis of *Ophiacodon*

between atlas and axis. Axis with anterior zygapophysial facets for articulation with atlas.

Interclavicle with expanded anterior part and a long posterior stem; clavicles large, smooth, articulating with interclavicle, scapula and sometimes with the cleithrum; scapula and coracoid suturally united, fused in maturity; anterior coracoid, the so-called procoracoid (the true coracoid of later reptiles) always forming a part of the glenoid articulating surface; a supracoracoid and a supraglenoid foramen always present; no ossified sternum. Pubis and ischium plate-like, the former pierced by a pubic foramen (rarely a notch), and both meeting their mates in a median

symphysis. Acetabulum imperforate, formed by all three bones; pubis, ischium and ilium more or less fused in adult.

Humerus expanded at its two ends into more or less divergent planes; an entepicondylar foramen present; olecranon more or less produced; radiale, intermedium, ulnare, centrale, and four distalia always ossified, a second centrale and the fifth distale rarely unossified; pisiform usually (perhaps always) present. Two bones in proximal row of tarsus (calcaneum and astragalus), at least one free centrale (rarely unossified) and five distalia. Manus and pes pentadactylate, the phalangeal formula 2, 3, 4, 5, 3-4; a vacuity for the mesopodial perforating artery in both carpus and tarsus (*Limnoscelis*?, *Diasparactus*?).

INCONSTANT OR VARIABLE CHARACTERS

Skull smooth or rugose, with two, one, or no temporal perforations on each side; rarely with spiny protuberances; postparietal, tabulare and supratemporal inconstant, primarily with all known membrane bones of the *Stegocephalia*; lacrimal usually extending to nares; transpalatine not yet demonstrated, quite surely absent in some forms; no posterior palatine vacuity hitherto observed; basisphenoid suturally or loosely articulated with pterygoids.⁴ Teeth usually thecodont, rarely acrodont, usually attached to vomers (prevomers), rarely to splenials. Occipital condyle convex, flat or concave distally.

From two to six or seven cervical vertebrae; twenty-three to at least twenty-seven presacral; one to three sacrals; and from about twenty-five to more than fifty caudals.

Articulation of ribs either continuous from capitulum to tuberculum, or separated by a distinct neck; that is, the ribs are either single-headed or double-headed. Spines of thoracic vertebrae rudimentary, or short, or moderately elongated, or greatly elongated. Ventral ribs present or absent.

⁴ Incidentally I may remark here that Versluis' recent identification (*Zool. Jahrb.*, 1912, 651) of the basisphenoid in the American pterodactyl *Pteranodon* is indefensible. The basisphenoid invariably forms part of the brain capsule, lodging the hypophysis. As Versluis would identify the bone in *Pteranodon* it is far removed from all relations with the brain-case or the cranial bones. Eaton, I believe, is right in restricting the basioccipital to the extreme posterior part.

Cleithrum well developed, vestigial or wanting; posterior coracoid bone (the so-called true coracoid bone) usually ossified, its suture more persistent than that between the scapula and anterior coracoid; sometimes unossified (*Seymouria*, *Varanosaurus*), a coracoid emargination, as in the *Lacertilia*, rarely present. Pelvis with or without a median puboischiadic vacuity.

Humerus rarely with an ectepicondylar foramen, usually with an ectepicondylar groove; terminal phalanges flattened or claw-like. A second centrale pedis rarely present. Articular extremities of long bones sometimes imperfectly ossified in the adult.

Herbivorous (*Casea*); insectivorous (*Seymouria*); carnivorous (*Dimetrodon*); or eaters of crustacea, and other invertebrates (*Diadectes?*, *Pantylus*, *Edaphosaurus*, et cetera).

Subaquatic, littoral, cursorial or climbing reptiles.

The above characters, both 'constant' and 'inconstant,' are drawn exclusively from the American forms. To include foreign forms will require some additions to the latter list: Aquatic, piscivorous reptiles, with elongated neck, posterior nares and other aquatic adaptations (*Proganosauria*); sclerotic plates in orbits, supracoracoid foramen a notch (*Paleohatteria*).

The foregoing characters are the chief ones upon which we must depend for the present at least, for the classification of the known Permocarboniferous reptiles. Doubtless when we shall have become more intimately familiar with the skull structure in more forms not a few others will be added.⁵

These characters would seem to be ample for the separation of the known genera into various orders; and they would be if

⁵ At the present time there is much doubt regarding the intimate structure of the temporal and posterior cranial region in nearly all the genera here under discussion. In only the *Captorhinidae* of the *Cotylosauria*, is the controversy nearly at rest. In this family (*Captorhinus*, *Labidosaurus*) it is now known that a postparietal, a so-called tabulare, a squamosal and a quadratojugal are present only. The skull-structure of no single theromorph is beyond controversy. As to the real homologies of the various bones of the temporal region of the reptiles we are no nearer the solution than we were a score of years ago; no one really knows what the homology of the mammalian squamosal is in reptiles. Eighteen names—twenty if we take into consideration the different usages of supratemporal and squamosal—have been applied to the four elements more usually called 'epiotic,' supratemporal, squamosal and quadratojugal, and, except for uniformity

we could distribute them at our pleasure. As a rule in taxonomy we depend not so much upon unique distinctive characters as we do upon different assemblages of characters common to various groups. A half dozen characters, if constantly associated in any given group would serve for its delimitation, the rank of the group being dependent upon the relative importance of the characters themselves. And the taxonomist is suspicious of any generic, family or ordinal classification based upon single characters. Rightly interpreted, such single characters, unaccompanied by other differences, may be and often are merely mutations or sports, unstable and of little significance. From which remarks it follows that I have no faith in the 'Mutation theory' of the origin of species, nor do I believe that any paleontologist can defend such a theory. And, frankly, neither do I believe that any theory of the origin of species, or of evolution even, can ever get very far when *time* is left out of account. If, in any series of phylogenetic forms we find a gradual transformation of structure, the gradual acquisition of new characters, we do right in uniting them all in a single group, for the sole end of all taxonomy is phylogeny.

Let us now attempt to define the two larger groups, the Cotylosauria and Theromorpha, in order to discover their distinctive or associated characters. Neither order has any single character to distinguish it from all other reptiles. The non-perforate temporal roof is shared between the Cotylosauria and Chelonia; the single temporal vacuity is common to both Casea or Edapho-

sake, one is as good as another. Huene, Case and I call the small bone on the posterior angle of the skull in the Captorhinidae and Procolophonia the tabulare ('epiotic'); others call it the supratemporal, and no one knows which it is. I at one time suggested the possible identity of the posterior arcade bone in the lizards with the tabulare, but no one seems to approve of it; and yet the small bone in the same position, and with like arrangements in the Procolophonia, Huene accepts as the tabulare. How little we really know of the homologies is evident enough in the fact that so acute an observer as Huene admits the possibility of the persistence of either supratemporal or tabulare. For the sake of uniformity solely, without committing myself to any theory of homology, I accept and use the terms supratemporal for the upper, squamosal for the lower element, wherever they occur, as in the Ichthyosauria, Lacertilia, Stegocephalia, Cotylosauria, etc.

saurus and the Therapsida; the double vacuities to Ophiacodon and the Rhynchocephalia, et cetera.

The characters found constantly in all members of either order are given in italics.

COTYLOSAURIA

Temporal region imperforate.⁶ Skull rugose or smooth; rarely with spiny protuberances. *Postparietals and quadratojugals always present. Lacrimals always entering into the posterior border of the nares. Teeth probably always on vomers (prevomers), rarely also on splenials; either thecodont or acrodont. Inter-temporal present or absent, as also either the supratemporal or tabulare, possibly both; primarily with all temporal bones of the Stegocephalia. Quadrate partly uncovered with a supratapedial process, or wholly covered. Posterior coracoid sometimes unossified. Neck short. Neural arches of presacral vertebrae stout; hyposphenes sometimes present; spines rudimentary or of moderate length; one or two sacral vertebrae; tail moderately short or long. Ribs single- or double-headed; abdominal ribs present or absent. Cleithrum well-developed, vestigial, or absent. No puboischiadic vacuity in pelvis. Ungual phalanges often obtuse, sometimes claw-like. Legs never elongate; ectepicondylar foramen never present. Rarely with supracostal dermal plates (Diadectes). From twenty-three to twenty-six presacral vertebrae.*

THEROMORPHA

One or two temporal vacuities on each side. Skull rugose (Casea) or smooth, rarely with spinous protuberances. Postparietals rarely (Edaphosaurus?) or never present, the tabulare

⁶ The Procolophonia have in no sense a perforated temporal region. I suggested several years ago (Biol. Bull., 1904, p. 166) that the uncovering of the temporal region in Procolophon was due solely to the extension backward of the orbit, and not to the fusion of supratemporal fossa and orbit. This view has been adopted by Huene (Paleontographica, 1912, p. 101) who would, however, give a distinctive term, pseudostegocrotaphic, to the condition found in this genus and its allies. But if this term be used with the Procolophonia, one must consistently use both terms, stegocrotaphic and pseudostegocrotaphic, for the conditions found in the living Chelonia, in Chelone and Chelydra, for instance.

never (?). Lacrimals usually not entering nares. Teeth thecodont (or protothecodont?), rarely attached to splenials or vomers (prevomers). *Neck of greater length. Neural arches never stout.* Ribs single- or double-headed, the cervical ribs sometimes dilated distally. Vertebral spines rudimentary, of moderate length or greatly elongated. Abdominal ribs present or absent. Two or three sacral vertebrae; tail short or of considerable length. Posterior coracoid bone sometimes unossified. Cleithrum well developed, vestigial or absent. Usually, but not always, a puboischadic vacuity in pelvis. Ungual phalanges rarely obtuse, usually claw-like. Legs usually less stout, sometimes very much elongate; ectepicondylar foramen rarely present. No dermal ossifications. Presacral vertebrae from twenty-four to at least twenty-seven in number.

The only characters, it is seen, by which an unknown genus may be referred with assurance to one or the other of these orders are found in the perforation or non-perforation of the temporal roof, and the neural arches of the presacral vertebrae. The stout neural arches are, however, not peculiar to the Cotylosauria since they occur in the Proganosauria. Nevertheless, the Thero-morpha are, as a whole, a more advanced group of reptiles than the Cotylosauria, as observed in the longer neck; longer, more ambulatory and prehensile legs, more slender bones, and especially in the constant reduction of the cranial bones. No thero-morph has all the cranial elements of Seymouria or Pantylus, and it is probable that none has all the elements found in Captorhinus or Labidosaurus even, perhaps the most advanced types of American Cotylosauria. And it is precisely in the skull structure that we may hope eventually to reach the most accurate comprehension of the interrelations of the two groups.

Ordinarily the differences known to exist between the different groups of the Cotylosauria—Diadectosauria, Pantylosauria, Pareiasauria and Procolophonia—might be considered of ordinal value if occurring among living reptiles. The Captorhinidae or Procolophonidae, for instance, have in the posterior cranial and temporal region the postparietal, tabulare, squamosal and

quadratojugal; they have no cleithrum; ventral ribs are present; and the thoracic ribs are single-headed.

The Seymouriidae, on the other hand, have all the cranial bones of the Stegocephalia, according to Broili and Williston, namely, the postparietal, intertemporal, supratemporal, tabulare squamosal and quadratojugal. They have no cleithrum nor ventral ribs; and the thoracic ribs are distinctly double-headed.

The Pantylidae, known only from the skull, have all the known stegocephalian roof elements of the skull, except the intertemporal; the teeth are acrodont, and are, for the most part, attached to broad palatal and splenial plates.

The other families of American Cotylosauria all possess a cleithrum, no ventral ribs, and single-headed thoracic ribs.

While these differences may seem to be important, it is very much of a question whether in such primitive reptiles they mean what they would in later and less plastic types. They do mean, as has been said by Case, that, even as early as the close of the carboniferous times, the most primitive reptiles known to us had already undergone many changes and divergences.

PROTEROSAURIA

Paleohatteria. The relationships of *Paleohatteria* with the American Permocarboniferous Theromorpha are, I believe, incontestable. Already such relationships have been suggested by Broili and Case, as well as others. Osborn, under the belief that *Dimetrodon* and the true pelycosaurs possess two temporal vacuities on each side, placed them in his superorder Diaptosauria; and, for a long while they were classed under the Rhynchocephalia in our text-books.

For many years, ever since its original description indeed, *Paleohatteria* has been accepted as either directly ancestral to or a member of the Rhynchocephalia. Baur united it with his Proganosauria, which he considered ancestral to all later reptiles. Nor can these relationships with the Rhynchocephalia, as first emphasized by Credner, be denied. In my contestation that the Proterosauria are really Theromorpha I am merely

going back to the first position held by Cope, as well as that held by Baur and Osborn. It may truthfully be said that it would not require a very great extension of the list of inconstant characters given on the preceding pages to include most of Osborn's Diaptosauria. I draw the greater dividing line elsewhere, that is, between the Proterosauria and Rhynchocephalia rather than between the Pelycosauria and Cotylosauria or Therapsida. I am proposing no new scheme of classification in the present paper; I am only endeavoring to show the futility of some of the present ones. Perhaps, when we know a great deal more about the older reptiles, we may be able to recognize a true phyletic classification, but I do insist that we have not that requisite knowledge at the present time, that the most we can do is to venture guesses, shrewd guesses perhaps, but still guesses, and that it is better to endure our present ills than to fly to others we know not of.

A minute comparison of the characters of Paleohatteria with those given under the preceding lists of the American Theromorpha would require too much space here, and is unnecessary. Suffice it to say that the diagnosis given by Credner (op. cit., p. 545) applies almost word for word, to the American forms with the exception of sclerotic plates in the orbits, which can be interpreted only as an aquatic character. Not only do the known characters agree throughout with those of the Theromorpha as a whole, but almost all of them will apply to the family Poliosauridae, and Ophiacodon and Varanosaurus especially; the first two paragraphs of Credner's diagnosis will, indeed, apply verbatim to Ophiacodon; and, so far as the characters of Proterosaurus from the upper Permian are known, there is little or nothing discrepant; but I omit their discussion here.

While it is very possible that Paleohatteria has two temporal arches on each side, the presence of the upper fenestra has never been positively proven. Credner in his first description merely says that the presence of the supratemporal fenestra is 'wahrscheinlich.' But its presence or absence is really unimportant in consideration of the fact that the two vacuities, both upper and lateral, are known to occur in at least one genus of American

Theromorpha; and the relations of some of the American genera, with and without the second fenestra, are so intimate that their family separation is very doubtful.

My conclusion then is that Paleohatteria and the Proterosauria can not be distinguished from the Theromorpha by ordinal characters, and should be united with them. I would provisionally classify the order as follows:

Order Theromorpha

Suborder Pelycosauria

Family Clepsydropsidae (Sphenacodontidae)

Family Poliosauridae

Family Edaphosauridae

Suborder Proterosauria

Family Paleohatteridae

Suborder Caseosauria

Family Caseidae

Suborder

Family Kadaliosauridae (Araeoscelidae)

I am aware that this classification is what might be called 'horizontal,' rather than 'vertical,' but I also insist that horizontal classifications are absolutely imperative until such time as we have more than vague surmises and guesses as to the true lines of phylogeny. Smith Woodard has pertinently criticised the prevalent fashion of making phylogenies to suit every passing hypothesis. And the fashion has found its climax in the 'phylogenies' of Steinmann, which I can only understand as proposed in an exquisite spirit of irony.

PROGANOSAURIA

The order Proganosauria was proposed by Baur in 1877⁷ to include *Stereosternum* and *Mesosaurus* only, founded chiefly upon the five distalia of the tarsus, a character unknown in modern forms, but one which we have seen is common to all known Permocarboniferous reptiles. Later⁸ he added *Paleohatteria*,

⁷ On the phylogenetic arrangement of the Sauropsida. *Jour. Morph.*, vol. 1, p. 103, 1887.

⁸ *Amer. Jour. Sci.*, vol. 36, p. 311, 1889.

to the order, dividing it into the two families Mesosauridae and Paleohatteridae, which he proposed, with the following definition: "Humerus with entepicondylar foramen; five distinct tarsal bones in second row, one for each metatarsal; pubis and ischium broad plates; each set of abdominal ossicles consisting of numerous pieces; condyles of limbs not ossified;" all of which will be found among the characters given for the American *Theromorpha* on the preceding pages. It was doubtless under this wider conception of the group, that Broom added to the order, hesitatingly, the genera *Saurosternon*, *Heleosaurus* and *Heleophilus* from the middle or upper Permian of Africa. These are rejected by Huene,⁹ and I agree with him. In 1892 Seeley proposed the ordinal name Mesosauria to include not only *Mesosaurus* and *Stereosternum*, but also some of the *Nothosauria*.¹⁰ Osborn in 1904,¹¹ restricted the term to its original limits and his example was followed by McGregor.

It is very clear that the name Mesosauria, so often used for the group, has no legitimate standing. Seeley and not a few others have believed that the Proganosauria are primitive *Sauropterygians*. Osborn and McGregor, on the other hand, would locate the group among the *Diapsida* and *Diaptosauria*, under the assumption that there are two temporal arches on each side, an assumption that has recently been denied by Huene (l.c.), who believes that the group is an independent offshoot from the primitive *Cotylosauria*, and more or less related to the *Ichthyosauria*. He says definitely that *Mesosaurus* has the supratemporal fenestra only, and that the lower arch is lost; as Jaekel declares is also the case with the *Sauropterygia*. There may have been and doubtless were, different ways in which the temporal fenestrations arose among *Reptilia*, but, with all due respect to the expressed opinions, I am not yet convinced that the explanations explain. I can conceive how a 'lateral' vacuity may have been converted into an 'upper' one or vice versa, or how the two vacuities may have fused into one, and I am not

⁹ *Paleontographica*, vol. 59, p. 100, 1912.

¹⁰ *Quart. Jour. Geol. Soc.*, 58, p. 586, 1892.

¹¹ *Mem. Amer. Mus. Nat. Hist.*, 1, p. 481.

yet ready to accept phylogenies and systems of classifications based upon such vague distinctions. Neither am I convinced that the quadrates of the Ichthyosauria and the Proganosauria are of such a distinct type as to require the phylogenetic union of the orders.

Case has expressed the opinion that "Mesosaurus is probably an aquatic adaptation of the Cotylosaurian type"¹² and I have also, independently, urged the relationships of the Proganosauria with the Theromorpha under the assumption of a single temporal vacuity.¹³ Nevertheless, the Proganosauria, in their aquatic adaptations are a definite group of primitive reptiles, of a distinct type, as shown in their elongated maxillae, posterior nares, elongated neck, long and flattened tails as figured by McGregor, short, fan-like scapula, elongated propodials and shortened epi-podials, et cetera. The vertebrae appear to be almost typically cotylosaurian in their thickened arches and low spines. The ribs are described as single-headed, attached below the diapophyses, but I have given reasons for a different interpretation elsewhere.

Aside from the distinctive aquatic adaptations, Mesosaurus and Stereosternum show primitive cotylosaurian or theromorph characters in the presence of teeth on the vomers (prevomers) deeply biconcave vertebrae, pectoral and pelvic girdles, entepicondylar foramen and ectepicondylar groove, carpus, tarsus, digits, et cetera. The aquatic adaptations of the limbs scarcely differ more from the terrestrial theromorph structure than do those of the dolichosaurs from the true lacertilians, and the aquatic modifications of the skull are of but little greater moment than those of the mosasaurs.

However, I am willing to consider the Proganosauria as a distinct order of reptiles until more shall be known about them.

REPTILES OF THE LOWER PERMIAN OF EUROPE

As an appendix to the foregoing discussion of the better known lower Permian and upper carboniferous reptiles, the following

¹² Revision of the Cotylosauria, p. 118, 1911.

¹³ American Permian vertebrates, p. 111, 1911.

brief notice of other less well known European genera will be necessary.

Phanerosaurus naumanii H. V. Meyer. The type and known remains of this genus and species consist of a series of four dorsal and a sacral vertebrae. They can not be distinguished from corresponding ones of *Diadectes*, and are doubtless truly cotylosaurian.

Stephanospondylus Stappenbeck. This genus was based upon the species *pugnax* Geinitz and Deichmüller, referred by its authors to *Phanerosaurus*. So far as the skull and vertebrae are concerned they seem to be genuinely cotylosaurian, but the pectoral and pelvic girdles, if correctly recognized and figured by Stappenbeck, are very aberrant, not only from the *Cotylosauria* but from all early reptiles. The clavicular girdle is unlike anything known among American reptiles, and seems more like that of a *temnospondyl*. The coracoid and pubis also are unlike anything known among early (or late) reptiles. I suspect that the so-called coracoid is in reality the pubis, and that the so-called pubis is something else, possibly a sacral rib. If it be really a pubis it indicates a pelvis of a radically distinct type, one with a large puboischiadic vacuity, as in modern reptiles.

Stereorhachis dominans Gaudry. The relationships of this genus with the American *Pelycosauria* in the narrow sense have long been recognized. Thevenin describes and figures the typical specimens and refers them to the *Pelycosauria*. None of its characters seems discrepant, so far as they are known. *Stereorhachis* comes from the lower part of the Autunian of France, possibly of contemporaneous age with the Clear Fork beds of Texas. It is associated stratigraphically with *Actinodon*, a genus closely allied to *Eryops*.

Calibrachion Gaudryi Boule. This genus was referred by Case;¹⁴ to the *Pelycosauri* notwithstanding the reputed opistho-coely of the cervical vertebrae, and the high coronoid of the mandible. Huene has since showed¹⁵ that both these characters were erroneously ascribed to the genus, though he objects to its

¹⁴ Revision of the *Pelycosauria*, 1907.

¹⁵ *Centralbl. für Mineralogie*, p. 534, 1905.

location among the Pelycosauria, rather than the Proterosauria. A detailed review of its characters is unnecessary here; the reader will find them in the cited work of Case or in the original paper. So far as I can discover, the genus presents no aberrant characters to distinguish it from the Theromorpha. The temporal region of the skull is unknown; both upper and lateral vacuities have been assigned to it under its assumed relations to Paleohatteria and Sphenodon. Possibly Baur's remarks may be applicable here, as so often elsewhere:¹⁶ "Dieser Fall zeigt sehr deutlich, wie leicht man sich täuschen lassen kann, wenn man durch eine allgemeine gültige Anschauungsweise beeinflusst wird." I doubt very much whether Callibrachion had both upper and lateral temporal fenestrae.

The remains of this genus, together with those of Haptodus and Sauravus cambryi come from the Gargenne schists of the upper part of the Autunian, whose position in the lower Permian I supposed was unquestioned. Huene, however, refers both Callibrachion and Haptodus, as also Aphelosaurus, to the 'oberen Perm,' but gives no reason therefor.

Aphelosaurus lutevensis Gervais. This genus and species are known from a single specimen from the lower Permian of France. The specimen lacks the head, neck and tail. No intercentra have been detected, though they are doubtless present. Slender abdominal ribs are present. The observed phalangeal formula of the hand is 2, 3, 4, ?, 3. So far as the published characters indicate, there is nothing in the genus to distinguish it from the American Theromorpha.

Haptodus baylei Gaudry. Thevenin, from a study of the known material, reaches the conclusion that the genus is identical with Paleohatteria Credner published several years later, but is inclined to think that the species are distinct.¹⁷ Haptodus, like Paleohatteria is known only from specimens lacking the ossific ends of the limb bones, which, taking into consideration the number of specimens studied by Credner, would seem to be sufficient proof that the character is an adult one. And such is doubtless the condi-

¹⁶ Zool. Anzeiger, Bd. 12, p. 239, 1889.

¹⁷ Annales de paleontologie,

tion in the American genus *Clepsydrops*. These two specimens of *Haptodus* add nothing to our knowledge of Paleohatteria, if they be the same, except that one is a half larger than those studied by Credner.

Kadaliosaurus priscus Credner. This genus, so far as I am aware, is represented by a single known specimen, from the middle Rothliegende of Germany, described and figured by Credner. The specimen, unfortunately, lacks the skull, the pectoral girdle and most of the tail, and has only stray bones of the feet. So far as it goes, as described and figured by Credner, it has a remarkable resemblance to *Araeoscelis* Williston, from the Clear Fork beds of Texas. It differs apparently in the possession of a well defined ventral armor, of which there is little or no evidence in *Araeoscelis*; perhaps also in having a short tail, though I should think it more probable that the tail is long, as in *Araeoscelis*. The vertebral spines in both are very short, the centra are notochordal, and the body is very slender. The resemblance of the limb bones in the two genera is astonishing; so great indeed that Credner's figures would serve equally well for *Araeoscelis*. Not only are the front and hind limbs of equal length, but the epipodials are as long as the propodials, and all are very slender. The femora of both forms are identical in their slenderness and sigmoid curvature. What is still more remarkable, the humeri, apparently agreeing quite in shape, have both entepicondylar and ectepicondylar foramina, a character unknown in any other reptile of lower Permian age. There is no puboischiadic vacuity in the pelvis of either genus. It would seem impossible that such extraordinary resemblances should be solely the result of convergent evolution. I believe that they are related and that both should be placed, for the present at least, in the same family. The family differs so much from others of the Permian-carboniferous that its relationships may well remain a matter of doubt. *Araeoscelis* has a single temporal vacuity of large size, and was a very slender, and slender legged, long tailed lizard-like reptile, almost certainly of climbing habits; its toes have acuminate claws.

Datheosaurus macrourus Schroeder. This genus and species from the lower Rothliegende of Germany, is unfortunately known only from a single specimen, not in the best preservation. It is a very slender reptile with a very long tail, with no known characters at variance with the preceding lists. Doubtless the interclavicle has an elongated stem and the humerus an entepicondylar foramen. Whether the temporal region is perforated or not is not known. In other characters, especially the structure of the vertebrae, it is sharply excluded from the Cotylosauria. Its nearest relationship seems to be with Kadaliosaurus, Araeoscelis and Paleohatteria.

MICROSAURIA

The name Microsauria was first proposed by Dawson in 1863¹⁸ based upon Hylonomus, a genus described by him from the carboniferous of Nova Scotia. Nearly thirty years later, Credner described¹⁹ more fully and in detail another species, *H. geinitzii*, from the middle Rothliegende of Germany, which he referred to the same genus, together with an allied new genus and species, *Petrobates truncatus* from the same horizon. In 1897, Baur²⁰ expressed the opinion that both forms were, in reality reptiles as Credner had suspected:

Die Frage ob Hylonomus und namentlich ob Petrobates den Stegocephalen oder aber den Rhynchocephalen zuzurechnen seien, lässt sich nicht durch ein kurzes Wort entscheiden. . . . Wenn man bei Petrobates vom Schädel absieht, welche nicht genau genug bekannt ist, so könnte man diesen Vierfüssler für einen kleinen Rhynchocephalen aus der Familie der Proterosaurien halten, wenn dem nicht das Vorhandensein von nur einen Sacral-wirbel entgegenstände.²¹

I scarcely doubt but that had Credner known of stegocrotaphic reptiles with a single sacral vertebra, such as *Diadectoides* and

¹⁸ "My first impression is that they constitute a separate family or order, to which I would give the name Microsauria, and which may be regarded as allied, on the one hand, to certain of the humbler lizards, as the Gecko or Agama, and on the other to the tailed Batrachians." Air breathers of the coal period, p. 47.

¹⁹ Zeitschrift der deutschen geologischen Gesellschaft, Bd. 42, p. 241, 1890.

²⁰ Anat. Anz., Bd. 14,

²¹ Credner, op. cit., p. 257.

Seymouria, he would have accepted both Hylonomus and Petrobates as reptiles. However, Baur's more decided views as the reptilian nature of the Microsauria were accepted by not a few authors, and their reptilian affinities, or at least those of some of the forms called Microsauria, have been recognized by all. Indeed, by some writers the Microsauria are considered the ancestral type from which some if not all reptiles arose. Nevertheless, they have been generally retained among the Stegocephalia, either under the ordinal or subordinal term Lepospondyli, proposed by Zittel, or the earlier Microsauria of Dawson. That the group Microsauria, as usually defined, is a heterogeneous one is now admitted, but sufficient evidence to serve as a secure foundation for its disintegration and the redistribution of its various forms on a scientific basis has not been forthcoming; and I doubt whether any proposed scheme will stand the test of time, or be worth serious consideration. The very recent scheme of classification proposed by Jaekel, in which he gives to the group a class value, dividing it into various orders, betrays such a regrettable lack of knowledge that it may be disregarded.

A few years ago M. Thevenin described a peculiar reptile-like form from the Stephanian or uppermost carboniferous of France as *Sauravus costei*, referring it to the Rhynchocephalia.²² In the following year I redescribed²³ an allied form which previously had been described and figured by Cope²⁴ under the name *Isodectes punctulatus*, from the Linton beds of Ohio, of middle Pennsylvanian age, referring it, together with *Sauravus costei* provisionally to the Cotylosauria. As a comparison of the specimen upon which Cope's remarks were based with the real type of the species showed specific differences at least, I gave to his plesiotype the name *Isodectes copei*, later changed to *Eosauravus copei*²⁵ when it became evident that the species could not be referred to the Texas genus *Isodectes*, of much

²² *Annales de paleontologie*, 3, p. 19, 1907.

²³ *Journal of Geology*, 16, p. 395, 1908; Moodie, *Proc. U. S. Nat. Mus.*, 37, p. 11, 1909.

²⁴ *American Naturalist*, 1896; *Proc. Amer. Phil. Soc.*, p. 88, 1877.

²⁵ *Bull. Geological Soc. Amer.*, vol. 21, p. 272, 1910; Case, *A revision of the Cotylosauria of North America*, p. 31, 1910.

later age. In the same and a later paper²⁶ I expressed the conviction that:

At least two distinct groups have been associated among what are called Microsauria, and that one of them, with single-headed intercentral ribs and intercentral chevrons, represented by *Hylonomus*, *Petrobates*, *Sauravus* and *Eosauravus* must be dissociated into a group more nearly allied to, possibly identical with, the Reptilia in a wide sense, while the other, of which *Urocordylus* may be taken as a type, may remain with the Amphibia.

For the former group I accepted the name Microsauria; for the latter, tentatively, Lepospondyli. In the same year Thevenin,²⁷ in a further discussion of *Sauravus*, with the description of an additional species, evidently reached a like conclusion, referring the genus to the Microsauria, taking *Hylonomus Dawson* as the type of the order, and placing it among the Reptilia. However, Dr. Moodie informs me that *Hylonomus Credner*, upon which most of our knowledge of the genus is based, is by no means certainly proven to be identical with *Hylonomus Dawson*, the real type of the Microsauria; that indeed the latter may not belong in the same group with the former, a conclusion not surprising, considering the different geological ages of the two. If such be the case, and it will not be possible to answer the question satisfactorily until more material referable with certainty to *Hylonomus lyelli Dawson* has been attentively studied, the terms, as I use them, are merely tentative.

Unfortunately the differential classification of the two groups is still chiefly dependent upon the skeletal structure aside from the characters of the skull, and, until the latter is better known than it is at present among the Microsauria (in the above sense) we lack a very important means of discrimination. For the present therefore I would distinguish the two groups, whether the Microsauria be reptiles or not, as follows (for the present I leave out of account the Diplocaulidae and Aistopodidae):

Lepospondyli (as typified by *Urocordylus*). Vertebrae holospondylous, notochordal; ribs elongate, the head not attached

²⁶ *Journal of Geology*, vol. 18, p. 599, Oct., 1910.

²⁷ *Annales de paleontologie*, 5, p. 43, 1910.

intercentrally; chevrons exogenous processes from body of vertebra; no intercentra; a single sacral vertebra; carpus and tarsus unossified; or, if ossified, a separate intermedium tarsi present; skull with two occipital condyles; parasphenoid well developed.

Microsauria (as typified by *Eosauravus*, *Sauravus*, *Hylonomus Credner* and *Petrobates*). Vertebrae holospondylous, notochordal; ribs long, attached intercentrally, usually without distinct separation into head and tubercle; chevrons intercentral, not exogenous processes from the centrum. One (?) or two sacral vertebrae; carpus and tarsus ossified; no separate intermedium pedis; phalangeal formula 2, 3, 4, 5, 4 in foot (*Eosauravus*, *Sauravus*). Interclavicle with long posterior stem. Skull (imperfectly known in *Hylonomus Credner* and *Petrobates Credner*) evidently stegocrotaphic; palate with small teeth; parasphenoid slender (in front at least). Small animals.

Intercentra and dorsal ossifications have been discovered in none of these genera while ventral ribs are known in all save the single known specimen of *Eosauravus copei*, in which they may have been lost in fossilization. Ossified mesopodials are known in all the genera, and apparently all have but the two bones, astragalus and calcaneum in the proximal row of the tarsus. *Hylonomus Credner* and *Petrobates* are from the middle Rothliegende of Germany, *Sauravus Thevenin* typically is from the uppermost carboniferous of France, and *Eosauravus Williston* is from about the middle of the Pennsylvanian of Ohio.

Taking these genera into consideration as a whole, it is seen that there is nothing yet known to differentiate them from the other primitive genera included under the definitions of the foregoing pages (unless it be the absence of intercentra); while, on the other hand, there are a number of characters, and those of considerable importance, that distinguish them from the Amphibia. It was some of these characters which induced Moodie to give an ordinal position to the Diplocaulidae, in which he was quite right, under the assumption that the Microsauria as a whole are a homogeneous group. But it is evident that, as usually grouped the Microsauria are not homogeneous; that some of the genera hitherto included among them are genuine amphibians, in all

probability allied to *Diplocaulus*, while others must be removed from the order.

But I care not whichever name is applied to the group I here call *Microsauria*, since in all probability it will be repeatedly changed before a fairly definite conclusion is reached; and that conclusion will not be reached until we know vastly more about the carboniferous vertebrate fauna than we do at the present time.

If the *Microsauria*, as typified by the above mentioned genera, are true, or real reptiles, what position in the system should they occupy? The vertebrae are purely holospondylous, and it is the universal belief that rhachitomous vertebrae are primitive; to assume the reverse is to rewrite the morphology of the vertebrae, and especially of the atlas of the Amniota; nor has there been a shadow of an argument so far produced to disprove the theory. The cleithrum has never been observed in any microsaur, and its presence in the *Cotylosauria* can only be explained as a direct inheritance from the *Amphibia*. Since neither the intercentra nor cleithrum could have reappeared after their one-time loss the probability that any known microsaur could have been ancestrally related to the later reptiles is very slight. If these microsaur are real reptiles or even indirectly ancestral to any modern reptiles, we must of course seek in early rocks their ancestral *Amphibia*. But we have so far no evidence whatever that *Eosauravus* and *Sauravus* may not actually belong among the *Cotylosauria*, where some authors would place them. It is true the vertebral arches of *Eosauravus* and *Sauravus* do not seem to partake of the peculiar structure of the true *Cotylosauria*, but, inasmuch as the postsacral vertebrae of *Seymouria* are of the ordinary slender type, the character, though a useful one, cannot be profound.

I believe that the cited genera at least are real reptiles—there is no known reason for including them among the *Amphibia*—but, until more is known about them than at present, a definite position in the system can not be given them. One may continue to call them, following Baur and Thevenin, true *Microsauria* as distinguished from the *Lepospondyli*; or he may, after Broili

and Case, locate them among the Cotylosauria, and still use the name Microsauria in its usually accepted sense. I can not believe that the time has come for a new system of classification and new names.

LYSOROPHUS

The history of the genus *Lysorophus* will be found in the various papers of the appended list²⁸ and need not be recounted here. Suffice it to say that Broili and others urge that it is a reptile; while Broom and I believe it to be an amphibian closely allied to, if not a member of the Urodela; Moodie thinks that it is a gymnophionian; and Case is confident that it is an amphibian, though doubtful of its urodelan affinities. The question here at issue is: Is *Lysorophus* a reptile? And, if so, to what group of the known reptiles is it most nearly allied? *Lysorophus* is known to occur in the Permocarboniferous of Illinois, Oklahoma and Texas. My knowledge of the genus is based upon abundant material, coming chiefly from the Clear Fork beds of northern Texas. I would characterize the genus and group as follows:

Lysorophus. Slender, *Amphiuma*-like in form; of from 8 or 10 inches to possibly 18 inches in length; with small limbs. No pineal foramen; no lacrimal, postfrontal, postorbital, jugal or quadratojugal bones; an unpaired supraoccipital bone, on either side of which are two distinct bones, variously identifiable as the squamosal and tabulare, or supratemporal; prefrontal (lacrimal?) articulating with nasal, frontal and parietal; orbits confluent with the open temporal region; quadrate ossified, suturally united with squamosal, projecting downward and forward; no temporal bars; palate with large subcranial plate, probably the parasphenoid, or parasphenoid and basioccipital; vomer with rows of teeth; two occipital condyles, their articular faces directed inward and backward; one pair of ossified

²⁸ Cope, Proc. Amer. Phil. Soc., p. 187, 1877; Trans. Amer. Phil. Soc., 16, p. 287, 1886. Case, Jour. Geol. 8, p. 714, pl. II, ff. 12a, b, c; Bull. Amer. Museum Nat. Hist., 24, 531; Revision of the Amphibia of the Permian of North America, 68, 141, 1911. Broili, Paleontographica, 51, p. 94, 1904; Anat. Anzeiger, 25, p. 586, 1904; *ibid.*, 33, p. 291, 1908; Zittel, Grundzüge der Paleontologie, 2d ed., p. 218, 1911; Williston, Biological Bulletin, 15, 229, 1908; Jour. Geology, p. 600, 1910.

ceratobranchials, and *four* pairs of ossified epibranchials. Atlas short, cylindrical, concave behind, its body composed of a single bone, that is not divided into odontoid and intercentrum, supporting the paired neural arch; closely united to skull without intervening cartilage. Vertebrae notochordal, the neural arches each composed of paired bones meeting in the middle line above, but not united, and without spines; with a prominent diapophysis on each side of the arch for the support of the (for the most part single-headed) ribs; no intercentra and no free chevrons. Limb bones small, without ossified articular ends, the mesopodials evidently unossified, and the girdles not yet recognized. No dermal ossifications.

If the foregoing characters be correct, and I feel quite sure they are, it will necessarily follow that, if *Lysorophus* be a real reptile, it must occupy a place all by itself as a separate subclass, without known descendants or antecedents. It is an established principle in paleontology that an organ or bone once lost never reappears in any descendants. We know of no reptile in which the lacrimal, postfrontal, postorbital, jugal and quadratojugal are all wanting; we know of but one order, the *Sauropterygia*, in which single-headed ribs have become purely neuracental in attachment, and surely no one will include *Lysorophus* in that order! Nor could it be genetically allied to the *Lacertilia*, because the *Squamata* have single-headed ribs attached to the centrum only, a more primitive reptilian condition to which the single headed neuracental ribs of *Lysorophus* could not have returned. And all *Lacertilia* have bones in the skull which are wanting in *Lysorophus*; in none does the prefrontal articulate with the parietal, excluding the postfrontal. That *Lysorophus* is widely remote from all other contemporary reptiles may be seen from the foregoing pages. The entire loss of the intercentra, not only from the cervical, dorsal and caudal vertebrae, but also from the atlas, is a character unknown in any Amniote vertebrate, but is the universal condition among amphibians. And so also, is the presence of four (not three) pairs of epibranchials. If, therefore *Lysorophus* be really a reptile, it had become so extremely divergent from all other known reptiles by the close

of carboniferous times that it must represent a group in itself equivalent to all others then living; and, inasmuch as it could not have left any known descendants, its claim to subclass rank is inopprobrious.

It has been claimed that the skull of *Lysorophus* is really moncondylar, that the condyle is in reality a tripartite one, in which the middle or basioccipital part was represented in life more or less by cartilage, or that the atlas articulated in fact with all three bones—the basioccipital and exoccipitals. I can not accept this interpretation of the structure. One of the skulls in the Chicago collection has the 'atlas' in close articulation with the condyles, with no interval to correspond to a cartilaginous continuation of the middle part. The atlas is transversely truncate in front in the middle, its angles articulating obliquely with the condyles. A second specimen, in which the atlas has been cleanly dislodged, has the condyles quite as they are figured in the cited paper of Case. I will admit that there are some features here which indicate an ossification of the basioccipital and its articulation in the middle with the front end of the atlas; but, under any interpretation of the structure, the mode of articulation with the atlas is very different from that known in any reptile. While the single condyle in some *Cotylosauria* may be flat or concave, in life it was rounded out by cartilage. In no reptile is the condylar articulation known to be concave, as is positively the case in *Lysorophus*, if it really articulates in the middle with the atlas.

The general resemblance of *Lysorophus* to *Amphisbaena* is conceded, as is also its resemblance to the *Gymnophiona*, and these resemblances have deceived both Broili and Moodie as to the real relationships of the genus. Both the *Amphisbaenia* and the *Gymnophiona*, as also *Lysorophus*, are or were burrowing creatures, either limbless or with small limbs, with a more or less pointed head, open temporal region, small eyes, and an elongated, oftentimes worm-like body. But these resemblances do not necessarily indicate genetic relationships of any of them, any more than do the resemblances between a porpoise and an ichthyosaur indicate genetic relationships. It is a curious fact that

the loss of the lower temporal arcade, and the open condition of the temporal region, as in these forms, the snakes, lizards and salamanders, seems to be correlated with a head resting more or less prone upon the ground.

In my opinion *Lysorophus* has no direct ancestral relationships with any modern vertebrates; that the Urodela, or even the Gymnophiona, began in such extremely *Amphiuma*-like forms in the carboniferous would be contrary to all our paleontological experience. The whole structure of *Lysorophus* demonstrates its aquatic habits, in the chiefly perichondral ossification of its bones; in the widely separated sutures of the skull, as urged by Miss Finney; in the large and doubtless perennially functional branchiae; and in the mode of its occurrence, in beds of immense numbers, coiled up and in undisturbed positions.

The ribs of *Lysorophus* are usually described as single-headed, attached to a prominent diapophysis; and this is true for nearly all of them, but apparently not for quite all. In what appear to be anterior ribs, those probably attached near to the head, there is a distinction into head and tubercle, separated by a short neck. Just how the capitular end is articulated has not been determined, though it appears to be attached to a facet immediately below the diapophysis; it almost certainly does not articulate intercentrally, nor has any centrum been found showing a parapophysial facet, such as characterizes the contemporary *Crossotelos*.

The occurrence of limb bones in *Lysorophus* seems assured. To determine if possible whether the first specimens of limbs found associated with skeletons of *Lysorophus* were accidental or not, I some time ago requested Miss Marian Finney, a student of paleontology in the University of Chicago, to make a careful search of the abundant material preserved in the collections. I give the results of her studies, which I can corroborate, in her own words, as follows:

THE LIMBS OF LYSOROPHUS

BY MARIAN FINNEY

In 1908 Dr. Williston discovered in a nodule containing a series of *Lysorophus* vertebrae and ribs limb bones which he believed belonged with them, but he was not sure that they might not have been accidental intrusions from some other small, undetected amphibian. To determine, if possible, whether *Lysorophus* really is a limbless amphibian or not, Dr. Williston requested me to study carefully the material of the collections of the University of Chicago coming from Texas. As a result of this study I am able to say with assurance that the evidence proves, I think conclusively, that *Lysorophus* does possess limbs, though it has been impossible to determine whether the limbs are the front or the hind, or whether indeed both front and hind.

The limbs in life were evidently loosely attached to the vertebral column, and were easily separated and lost; and the bones themselves are so minute that, once separated from the skeleton they would hardly be detected, or if detected correctly recognized. Of the two hundred nodules examined, containing more or less of the skeleton of *Lysorophus*, I find fifteen containing limb bones, the most of them isolated; only a few with more than one bone associated.

The best of these specimens consists of a femur, tibia, fibula, two metatarsals and two phalanges all related. I assume that the limb is the hind one because of the shape of the bones only. The femur is 10.5 mm. in length, hollow throughout, with a rather slender shaft, and moderately expanded ends; its ends are mere shells of thin bone, easily crushed. The tibia and fibula are 6.5 mm. in length, or about three-fifths the length of the femur and, like it, are hollow. The tibia is thickset, the ends less expanded than those of the femur. The fibula is slender, and lies close beside the tibia. Beyond these epipodials are two bones which doubtless are metatarsals, though not much shorter than the tibia and fibula; they measure 5 mm. in length. Beyond these are two small bones which seem to be phalanges, lying one at the end of the other, each about 1 mm. in length.

There is no trace of mesopodials, and, in all probability, judging from the imperfect ossification of the leg bones, the tarsus was unossified. The limb lies in the matrix beside a long coiled series of vertebrae, but I can discover no trace of girdle bones.

Another specimen is not so complete and is somewhat larger. The femur (?) is about one-third longer, and is more massive, though hollow like the others, and with truncate ends. At or near the extremity of this bone lie four others, each about half the length of the femur, which probably are metapodials. This leg is associated in the matrix with several disconnected *Lysorophus* vertebrae, but with no trace of girdle bones.

Most of the numerous single bones found seem to be propodials, similar in character to the ones I have called femora, and of about the size of the larger one I have described. As a rule only isolated bones have been observed, and always in nodules containing vertebrae and ribs, and with no indications of other forms than *Lysorophus*. With one specimen a skull was found, but not connected with the vertebrae. In one of the nodules are two bones, apparently epipodials, in articulation with a fragment of a larger bone, a propodial probably; they are 6.5 mm. in length, or quite the size of the first specimen described. The larger of the two may be the tibia, the smaller the fibula. Associated with these two bones are parts of two lower jaws, measuring 7 and 8 mm. in length; in the larger fragment I count eleven teeth; they are sharply pointed, conical and recurved; and all these bones lie associated in the matrix with typical *Lysorophus* vertebrae only.

In general appearance *Lysorophus* must have resembled *Amphiuma* means very much. It had a slender body and slender tail. In one specimen I count eighteen vertebrae in an apparently complete tail; in another I count twenty in a gradually tapering series. That *Lysorophus* had legs there seems to be no longer doubt, but they evidently were not of much functional importance, since they are relatively very small. The total length of the body may perhaps have varied from 10 to 15 inches; a complete leg could not have been more than $1\frac{1}{4}$ inch in length. Evidently *Lysorophus* lived in pools or ponds of water, burrowing

perhaps in the soft mud of the bottom. They are found almost invariably coiled up, much after the manner of the modern *Amphiuma*. That they were burrowing in habit seems very probable because of the shape and structure of the skull, resembling much those of the *Amphisbaenia* or *Gymnophiona*, but the very loose sutures of the skull would hardly be adapted for burrowing in firm earth, after the habits of the lizards and blind worms, which have closely united skull bones. Not only this skull structure, but the mode of occurrence in immense numbers, closely associated together, as reported by Dr. Williston, and closely coiled, and especially the well ossified branchiae, seem to prove that *Lysorophus* was a perennial water breather.

University of Chicago, May 5, 1912.

THE PAEDOGAMOUS CONJUGATION OF BLEPHARISMA UNDULANS ST.

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TWENTY-FIVE FIGURES

The genus *Blepharisma* was created by Perty in 1849–1852 with the following diagnostic characters: Body flat, lancet-shaped, pointed posteriorly and ending anteriorly in a short snout (Schnabel); the deeply incised region extending from the anterior end to about the middle of the body is provided with a row of long straight cilia arranged in parallel lines. 'Molecular rows' bearing extremely fine cilia, difficult to see, are borne by the remainder of the body. Two species were described as new, *B. hyalinum*, a colorless form, and *B. persicinum*, a reddish colored form. The latter he regarded as possibly the same as Müller's *Trichoda striata*.

Ehrenberg ('31) in the meantime described a form under the name of *Bursaria lateritia* which he identified as the same as Müller's *Trichoda ignita*, having a distinct reddish color, compressed body and with a characteristic shape of a garden knife.

Stein ('67) described the genus in detail, including as synonyms Müller's *Trichoda striata*, *aurantiaca*, and *ignita*, *Bursaria lateritia* of Ehrenberg, and *Ypsistoma* of Bory, and characterized two species as follows:

1. *B. lateritia*: Body peach color, purple-red or tile-red, occasionally colorless. The peristome reaches as far as the middle of the body or beyond this point, with a free-standing bristle-like undulating membrane attached close in front of the peristome angle. The nucleus is a single oval body in the anterior half of the organism.

2. *B. undulans* St. The color is purple red; the peristome does not reach to the middle of the body but is usually limited to the anterior third, with a well-developed undulating membrane attached to the entire posterior half of the left peristome edge. One nucleus in the anterior half and one in the posterior half, with or without a connecting strand of nuclear material.

Stein maintained that Perty's two species were neither new nor distinct from one another, and interpreted Perty's 'molecular rows' as the body stripings. Stein's *B. lateritia* was more frequently seen, *undulans* only a few times and then in connection with numbers of *lateritia*. Bütschli regards Stein's *undulans* as the same as Ehrenberg's *Uroleptus musculus*, apparently ignoring the difference in the undulating membrane of Stein's two species, but accepting Stein's characteristics as given for *B. undulans*. Stokes, finally, has apparently described the genus under the name *Apgaria*.

While there is little difficulty in deciding upon the generic name *Blepharisma*, the task is not so easy with the type species. Stein has shown that Perty's two species cannot hold, while the descriptions of Stein's own species, careful and detailed as they are, cannot be traced back to any one type of Ehrenberg or Müller. Both his *lateritia* and *undulans* could be included in Ehrenberg's *Bursaria lateritia* which in turn, might go back to Müller's *Trichoda aurantiaca* and *T. ignita*. Neither Müller's nor Ehrenberg's descriptions of these species were sufficiently accurate to be recognizable today; similarly with all other characterizations save those of Stein, where we have to decide as to the validity of the two species *lateritia* and *undulans*.

The essential differences in structure between the two species given by Stein are in connection with the undulating membrane and the macronuclei. In *lateritia* the undulating membrane is said to be a mere bristle or cirrus-like structure inserted near the mouth in the angle of the peristome, while in *undulans*, the undulating membrane is much more extensive, extending half way up the peristome left edge. The bristle or cirrus was, probably, only the proximal edge of the undulating membrane.

The nuclear differences are less important and have no specific value as will be shown in the following pages.

The organism with which we are dealing in the present paper, belongs to Stein's second species, *Blepharisma undulans*, with the following characters: Size of longest individual observed 220μ , shortest, 70μ . Color variable, from deep purple violet to light rose, or perfectly colorless. Form variable according to the condition of the posterior end, but is usually sufficiently striking to be easily identified. It is somewhat lancet formed, thickest in the middle and tapering towards the two ends (fig. 1). The posterior end is usually broader than the anterior, and, save in exceptional cases, does not come to a point but ends in a broadly rounded surface. The anterior end, on the contrary, comes to a rounded point in which three lines converge, the left margin of the body, and the right and left borders of the peristome. The peristome is a deep cleft on the right ventral side of the body running and deepening slightly in an oblique direction towards the left (fig. 2). The adoral zone, composed of long slightly tapering membranelles, begins on the left of the anterior tip and is continued posteriorly on the left peristome margin to the mouth where it sinks below the surface with the peristome. An undulating membrane arises close to the mouth deep in the peristome and extends along the right margin of the peristome and towards the anterior end, stopping half way between the mouth and the end. It is about one-half as broad as long and extends well beyond the surface of the body so that it can be seen distinctly with the lower powers of the microscope (fig. 3). A second undulating membrane, much smaller and very difficult to see, even with the highest powers, extends from the mouth along the left margin of the peristome inside of the adoral zone, for a distance equal to about half that of the right membrane. This is probably the same thing as the 'lip' described by Anigstein in *B. clarissima*. Unlike *B. lateritia* the body, as a rule, is only slightly if at all compressed, but at times it is definitely flattened especially in the oral region. The body stripings are distinct and from nine to sixteen in number. The macronucleus is single, double or multiple, and free micro-

nuclei are absent. The contractile vacuole is terminal and is usually accompanied by one or more fluid vacuoles. Very often the entire posterior part is drawn out into a fine vacuole-filled thread which gives to the organism a striking resemblance to *Spirostomum tères*. Not infrequently this entire posterior vacuole-bearing part is pinched off, leaving the remainder of the body with a decidedly truncate base, recalling Müller's *Trichoda aurantiaca* (fig. 9).

Habitat and food

Müller found his *Trichoda ignita* among *Lemna* in fresh water, Bory found *Ypsistoma* on *Oscillaria*, and Ehrenberg, *Bursaria lateritia* on *conferva*. Stein also speaks of it in connection with filaments of algae. Bütschli speaks of its food only in a general way as 'Nahrung fein.'

I found a large number of specimens among decaying algae in pond water at Woods Hole, Massachusetts, in July, 1911. One individual was isolated in filtered water from the same source, and experiments were immediately begun to determine the best medium for its continued cultivation. Without going into details in regard to these experiments it will suffice here to say that the most satisfactory medium was found to be pond water with about 50 per cent of twenty-four hour hay infusion. On this medium which is renewed daily, the descendants of the originally isolated specimen have divided 224 times up to the present day (June 12), or less than one division per day on the average. All specimens have been colorless for the last five months. The food, therefore, appears to be bacteria of ordinary hay infusions, the best results being obtained with a relatively small number of bacteria, or in other words, with as little as possible of the bacterial waste matters.

Later in the summer (September) an effort was made to find other wild specimens from the same source as the original but without success, so that the observations here recorded were made on material derived from the original specimen isolated on the 20th of July. The full history of this culture, to-

gether with experiments on the effects of chemicals of different kinds, will be published in another place. In the present paper I desire only to present the observations on division and paedogamous conjugation.

Division of Blepharisma undulans

When conditions are fairly uniform in the cultures *Blepharisma* divides about once in twenty-four hours. No regular rate, however, is characteristic; at one period during the earlier stages of the culture the organisms divided as often as three times in one day, but later the division rate fell as low as two divisions in ten days.

When ready to divide, the cells appear slightly swollen in the middle and a new peristome appears in the posterior half of the cell (fig. 4). The individuals become sluggish and, even before the adoral membranelles are visible, a contractile vacuole appears immediately posterior to the mouth and in the most swollen portion. The membranelles of the posterior adoral zone first appear as cilia slightly more conspicuous than the cilia on the general surface. At this time there is no trace of the posterior peristome. A shallow furrow soon appears, however, and this gradually deepens, while the membranelles grow in size and vibrate with a rhythmic motion from anterior to posterior (fig. 5). The division furrow begins immediately posterior to the central vacuoles, below the point of maximum swelling, and then cuts in from the ventral surface towards the dorsal. Throughout this period I have been unable to see the conspicuous undulating membrane on either half of the dividing organism. On the posterior cell it does not appear until the mouth breaks through in the last five minutes of division. In the anterior half I have watched its vibrations up to the time of the beginning of constriction, when it could not be seen again until the end stages. Whether this anterior membrane is thrown off and another formed to replace it I cannot say. Possibly the mouth being closed at this period of division, the membrane is folded down on the floor of the peristome and is thus

out of sight. During this middle period of division the organism remains quiescent or moves but slightly, but as the end stages come on it moves more freely and with greater rapidity, both undulating membranes in active motion. Not only are new membranelles formed in the posterior half but the old membranelles of the anterior portion are also replaced by new ones (fig. 6). The new membranelles appear as minute cilia within the old row and grow quickly, moving at first with a spasmodic activity quite different from the regular wave-like motion of the older membranelles. As growth of the new membranelles progresses the regularity of motion increases until two full sets of membranelles are in play. This condition lasts but a few minutes, however, as the older set begins to fade away by absorption until finally replaced by the active new set.

None of the older observers was able to make out a micronucleus in the ordinary vegetative stages, nor during division, but Bütschli observed two 'encapsulated' smaller nuclei during the process of conjugation of *B. lateritia* which he regarded as micronuclei. Balbiani ('60) also is said to have observed the micronucleus in *B. lateritia*. Bütschli remarks on the difficulty experienced by himself in demonstrating the micronucleus and states that he was unable to identify it in the ordinary vegetative forms, although he did not doubt its presence in the ordinary cells, since he found it in multiple number in conjugating specimens.

The difficulties in demonstrating the presence of micronuclei have in no wise decreased with the lapse of time since the observations of these gifted pioneers. Different methods, however, and especially the use of sections, have enabled us to trace these elusive bodies and to get some light on their history, although it must be admitted that this history does not correspond with that of any other known ciliate. They are intimately associated with the macronucleus, and in resting phases of the cell I have failed to find positive evidence of their presence. During division, however, they appear as extremely minute, deeply-staining bodies which apparently divide within the nuclear membrane of the macronucleus (fig. 8). In total

preparations of the dividing cell it is possible to get them in profile, when they appear associated with the macronucleus very much as do the micronuclei of ordinary ciliates like *Paramecium* or *Colpidium*. The relations come out best in sections and especially sections of late stages in division. In early stages they are apparently within the nuclear membrane and are difficult to distinguish from the thick, granular chromatin of the macronucleus. Here and there, however, in the mass of chromatin, larger and more definite deeply staining bodies may be seen which may be the micronuclei (fig. 3). In later stages they become much more definite, although still small and inconspicuous. They lie close against the chromatin of the macronucleus and within the delicate nuclear membrane surrounding this organ. In some cases they are separated by a slight space, but this condition is exceptional. Ordinarily they lie in pairs (fig. 8), the highest number that I have seen being eight, or four pairs. The remarkable significance of this intranuclear position comes out in connection with the phenomena of conjugation to be described later.

The macronucleus, usually so definite in form and simple in its changes during vegetative life and division, is extremely difficult to interpret in the case of *Blepharisma undulans*. Stein described *B. undulans* as having two macronuclei, one in each half of the cell and with or without connecting chromatin threads. This condition is fairly common, but so also is the uninucleate condition and the multinucleate condition, while intermediate phases connect these two extremes. The single macronucleus is compact and homogeneous and may or may not have the characteristic 'Kernspalt' common to many of the ciliates. A common occurrence is the presence of two or three macronuclei grouped together as shown in figure 1, and in these cases there is a difference in texture between the different elements. I am inclined to interpret these as incomplete stages in the fusion of the portions of the macronucleus after division, for the nucleus during division always begins as a unit, stretching out as a single strand and breaking up into numerous portions at the end of division (figs. 2, 7, 8). The difference in texture

of the different portions is enigmatical and I have no suggestions to offer in interpretation. While the majority of the macronuclei are deeply staining, granular and uniform in texture, this one portion is much less granular, stains lightly and is more homogeneous than the denser portions. In some cases, furthermore, there is no such difference between the different portions, hence it may be only a transient or even a degenerate condition. Opposed to the latter hypothesis is the fact that the same texture is noted in the new nuclei formed as a result of conjugation. In one case this lighter portion was found at the extremities of the elongated macronucleus in division, where it appeared something like the centrosphere of *Noctiluca* in division but in other division figures there was no such differentiation.

In division, the central portion of the elongated strand becomes thinner until it finally breaks in the middle. The daughter nuclei do not round out then into definite homogeneous nuclei, but the reconstruction is accompanied by a fragmentation of the macronuclear material into from two to eight separated fragments of diverse size. Some of these portions are extremely small, and when rounded out, have the appearance of micronuclei, but no two cells are alike in this respect, a fact which, together with their mode of origin, contradicts any interpretation as to their micronuclear nature. The chromatin of the macronucleus is finely granular and has the tendency to collect about the periphery of the elongated strand, leaving the central portion lighter, thus giving the impression of an intranuclear canal. In one case the cell was of giant size, with a macronucleus in the reconstruction stage of division, although there was no evidence of division of the cell body (fig. 2).

The end stage of division is characterized by the peculiar twisting movements observed in so many ciliates, the last connecting strand of cortical plasma finally giving way and freeing the two cells, each now with its full complement of cellular organs, peristome, nuclei, and contractile vacuoles.

Occurrence and phenomena of conjugation

O. F. Müller (1786) described the conjugation of *Trichoda aurantiaca* and gave a figure that is easily recognized as a species of *Blepharisma*. Stein described a number of individuals which he regarded as ex-conjugants, but did not see the conjugating cells and drew an erroneous conclusion as to the method of fusion of the two individuals. Bütschli ('76) was the first to describe the process of conjugation in *B. lateritia*, making out the method of fusion of the two individuals and the presence of the micronucleus. An interesting feature of this work is that ex-conjugants were unable to live, many individuals dying on the second day after separation, the remainder on the third day.

Unfortunately I have been unable to study the exogamous conjugation of *B. undulans*, owing to the failure to find other specimens than those originally isolated. But paedogamous conjugation occurs constantly and the following account refers to that alone.

All who have carried on careful cultures of protozoa, isolate individual cells each day and transfer them to fresh culture medium, while the remainder are kept together in larger dishes (I use Syracuse watch glasses) and allowed to accumulate in large numbers as 'stock.' Shortly after beginning observations on *Blepharisma* it was noticed that conjugating individuals were frequent in such stock, amounting in some cases to regular epidemics. No degree of relationship seems too close to prevent such union, in one case, for example, an individual was isolated in the 104th generation of the race; it divided during the night and on the following day the two daughter cells were firmly united in conjugation. This, I believe, is the closest case of paedogamy in ciliated protozoa on record.

The externals of conjugation of *B. lateritia* as described by Bütschli agree fully with those of *B. undulans*. The two individuals unite, first at the anterior tips, as in the case of *Paramecium caudatum*, and then fuse along the somewhat spirally running central line of the peristome. Stein's assumption that

the edges of the peristome fuse is erroneous, while Bütschli was right in stating that both margins of the peristome are free (fig. 17). It thus happens that the adoral zone of one individual overlies the line of fusion on one side, while the adoral zone of membranelles of the other individual covers the line of fusion on the opposite side. Fusion, furthermore, does not extend posteriorly as far as the mouth but involves only about three-quarters of the peristome, leaving the mouth and a large portion of the undulating membrane free (fig. 14). *B. undulans* is never very active but during the process of conjugation it is even more quiescent than usual. The individuals remain united for a period of from eighteen to twenty-four hours, rarely longer than this, and finally separate. The process of separation begins with the vacuolization of the zone of fusion (fig. 22). The walls of the central vacuoles break down, leaving the two cells connected at the anterior tip and at the posterior point of fusion. The latter connection breaks first and the organisms finally separate at the anterior tips (fig. 21).

The conjugating population is distinctly smaller than the non-conjugating, a fact easily noted by the eye. Statistical confirmation of the size differences has been made by Miss Watters, whose paper on the subject will shortly appear, and she permits me to summarize her results to date as follows: 482 conjugating and 1089 non-conjugating individuals were measured, the average length of the former being 110.08 microns, and of the latter (including ex-conjugants) 140.37 microns. The longest conjugating individual was 156.25, non-conjugating 221.35 microns; the shortest conjugant was 85.93 microns, non-conjugant 70.31 microns. There can be little doubt that the shortest non-conjugant was an ex-conjugant, for these are always minute.

Stein believed that small forms with numerous nuclei were ex-conjugants, but Bütschli regarded such forms as abnormal specimens, not necessarily ex-conjugants. Under strictly normal conditions the nucleus may be multiple in *B. undulans*, without indicating any connection with conjugation. The smaller size of the suspected ex-conjugants and the evidences

of degeneration, which in Bütschli's and my experience follows conjugation, make Stein's interpretation more plausible.

Bütschli's observations were more complete and more definite than Stein's although the finer details are still unknown in *B. lateritia*. A free translation of his description of the nuclear changes runs as follows:

The uniform and finely granular (macro) nucleus as shown by treatment with acetic acid, undergoes no changes during the entire process of conjugation, and passes on to the separated animal in its original form. In flattened conjugating animals treated with 1 per cent acetic acid on the other hand, one notices very distinctly, a number of small nucleolus (micronucleus)-like bodies. These give the impression of small cell nuclei which appear as though enclosed within a dark membrane and consist of a dark central or ex-centric granule. Once fine fibers were seen running from this central granule (karyosome) to the membrane, and again one finds these vesicles sometimes without a central granule (karyosome) but composed of fine particles. The number of such bodies found in the cells of conjugating animals varied within rather wide limits; once there were only two, again three, seven, eight, while in one pair the one individual contained eleven the other one six. After conjugation there is no further evidence of these bodies; on the other hand in a specimen that had just finished conjugation there was one very distinct nucleolus capsule in front and one behind the nucleus (macronucleus). The capsules were beautifully striated with two dark staining bodies in the centers of each fiber, which together formed a double granular zone in the equator of the capsules.

In place of these two undeniable nucleolus capsules one finds in animals shortly after conjugation two minute pale bodies of oval form on the side of the nucleus. These, after treatment with acetic acid, show large dark granules. They grow rapidly into pale round spheres in which an ex-centric granule is invariably present. In contrast to this development the nucleus (old macronucleus) becomes progressively smaller and denser and shows unmistakable signs of degeneration.

On the third day after completed conjugation, animals were found in which there was no trace of a (macro) nucleus and there is no doubt that the degenerated nucleus had been thrown out of the cell. The new light spheres continued to enlarge and appeared to be made up of small granules while the larger bodies had apparently disappeared (l. c., pp. 315-316).

All of the stages observed by Bütschli I have been able to duplicate in the case of *B. undulans*, and in addition have been able to trace out the beginnings and to a certain extent the his-

tory of the 'capsules.' The first statement, however, in regard to the unchangeableness of the macronucleus cannot hold for *B. undulans*. On the contrary, this is the very center of nuclear activity, and its early changes initiate the internal processes of conjugation. These early changes are characterized morphologically by three conditions of the macronucleus, not present at other times: (1) all macronuclear material is concentrated in one definite oval macronucleus; (2) this oval nucleus is enclosed in a thick doubly refractive cyst-like membrane; (3) the cleft or Kernspalt which appears occasionally in the vegetative nucleus, is here continued into a clear hyaline space which separates the nuclear material into two distinct parts.

The material of the macronucleus at this stage is condensed into a firm, massive nucleus with a characteristic appearance quite different from its appearance at other periods. The cyst-like membrane has not the least resemblance to nuclear membranes as usually seen. It has a distinct double contour and is separated by a clear space from the chromatin contents, and if seen free from the surrounding cell, would give to the macronucleus the appearance of an encysted flagellate (fig. 12).

The early changes of the micronuclei have not been seen, that is, their emergence from the macronucleus. Until fusion is completed they remain comparatively small and difficult to see, showing no such enlargement as do the micronuclei of *Paramecium* or other ciliates in which conjugation has been worked out, and staining with the greatest difficulty. In resting cells the characteristic number appears to be four. In conjugating cells there is often, but not always, a fifth micronucleus-like body which takes no part in the processes accompanying conjugation (figs. 12, 18 and 20). It degenerates and ultimately disappears. It may be an extra vegetative micronucleus, since the number of these nuclei varies in the vegetative cells; or it may be a residual degenerating nucleus from a first maturation division of the four nuclei, an interpretation which would bring the maturation phenomena of *Blepharisma* in accord with the phenomena in other ciliates. On this interpretation there would be three other similar nuclei to account

for, and it seems hardly probable that they, or the spindle figures giving rise to them, should have been overlooked. On the other hand they are so minute and so difficult to stain that this possibility must remain open.

The first evidence that I have found as to maturation divisions is the late stage in mitosis of the four functional micronuclei (fig. 12). They lie close together in the anterior third of the cell, the daughter nuclei appearing first as homogeneous chromatin then as vesiculate nuclei. One of the eight resulting nuclei divides again, forming the two pronuclei, while the other seven swell, degenerate and finally disappear (fig. 14). The two pronuclei are practically the same in size and appearance, although in one instance the two migratory nuclei were larger than the stationary (fig. 13). At the time of fusion the pronuclei are elongate and spindle formed, union taking place first at the ends (figs. 14 and 15). After fusion the syncaryon becomes spherical and the chromatin is uniformly distributed (fig. 16).

The interchange and fusion take place at the posterior limits of the zone of coalescence and the fertilization nuclei remain in this region during their further changes. Each nucleus forms a spindle and divides by typical mitosis (figs. 18 and 19), the full spindle figure (fig. 19) being much larger than any of the maturation nuclei and showing typical fibers and chromatin. The two nuclei in each cell resulting from this first division do not pass immediately into the second spindle stage but evidently remain for some time in the resting stage, since they are often found in various positions relative to the old macronucleus (figs. 21, 22 and 23). Each nucleus then divides again by mitosis, this being the stage described by Bütschli.

The period of nuclear quiescence after the first division is characterized, in the majority of cases, by the commencement and completion of separation of the conjugating cells. This is accomplished by the progressive vacuolization of the protoplasm in the line of fusion. The animals separate, first in the center of the fused areas by coalescence of the vacuoles, then at the posterior or oral region, and finally at the anterior tips.

In the meantime the old, encapsulated macronucleus undergoes fragmentation, the process showing no time relations in regard to the changes of the micronuclei (figs. 13 to 23). Sooner or later the fragments show a well-marked granular degeneration (fig. 14) and ultimately disappear, probably by absorption in the protoplasm.

The four daughter nuclei formed by the second division of the fertilization nucleus next show characteristic changes leading to the formation of the new macronuclei. They appear as deeply staining granules surrounded by a more feebly staining homogeneous matrix. The latter increases remarkably in bulk, probably by elimination of chromatin material from the micronucleus, until it attains the size of the macronucleus in vegetative cells (figs 24 and 25). These are the 'pale round spheres' which Bütschli described in 1876. In them, either central or ex-centric, are the micronuclei which are occasionally found in the process of division (figs. 24 and 25). These 'pale round spheres' are the new macronuclei. The further history of the nuclei of the ex-conjugants is unknown. The organisms rapidly grow smaller and ultimately die without division.

DISCUSSION

The structures and activities of *Blepharisma undulans* bring up again some large questions in protozoology. Of these, two in particular demand further consideration, viz., the origin and significance of macro- and micronuclei, and the effects of conjugation.

There is a strong probability that *B. undulans* and *B. lateritia* are one species, since the nuclear and peristomeal structures of *undulans* are so variable. In both types the micronuclei have hitherto been overlooked in the vegetative stages. *Blepharisma clarissima* Anigst., on the other hand, appears to be a distinct species with a characteristic beaded macronucleus and numerous micronuclei visible in the vegetative stages. The contractile vacuole, also, with its feeding canal is a specific characteristic.

The failure of previous observers to find micronuclei in *Blepharisma undulans* in the vegetative stages is explained by their formation and retention within the macronuclear membrane. Lebedew ('08) describes micronuclei emerging from the macronuclei during conjugation of *Trachelocerca phoenicopterus*, and the observations of Neresheimer ('08) show that some analogous process occurs in the parasitic ciliate *Ichthyophthirius*. The latter observations seem to be inconclusive, and the possibility at least is open that the new micronucleus does not actually penetrate and enter the old macronucleus, but gives rise to a new macronucleus as in *Blepharisma*, by secretion. In this connection Neresheimer says:

The most remarkable thing after encystment is the origin of the micronucleus (Nebenkern), which up to now has invariably been seen in young specimens without any inkling as to where it comes from. It appears suddenly and in a most peculiar manner, when from twenty to thirty fragments are present in the cysts. In sections of this stage, on the inside of the now spherical nucleus, there is a small, highly staining granule which appears like a comet with a tail of plastin and chromatin streaming out behind, connecting it with the nucleus. Soon after, this connecting strand disappears and the complete micronucleus takes a position some distance from the macronucleus.

Neresheimer does not say that it emerges from the macronucleus, but one certainly gets the impression that it is thrown out of the macronucleus. From now on the micronucleus divides with a typical mitotic figure at each division of the cell, while the macronucleus divides by direct division. Ultimately the micronucleus divides twice in succession; three of the four resulting nuclei degenerate, the fourth divides again to form male and female pronuclei characteristic of the ciliates. Conjugation occurs not between two cells, but by union of the two nuclei thus formed and the syncaryon then wanders into the old macronucleus where it remains for a time as a karyosome and finally disappears.

While it is probable that the micronuclei in these young forms emerge from the nucleus, we need further evidence than Neresheimer brings forward to support the view that the syncaryon penetrates the old macronucleus instead of forming a

new one by division or by secretion. In *Blepharisma* the new macronucleus is entirely different in texture and appearance from the old and there is no doubt of its new origin.

In *Blepharisma* then, we find quite a different process of nuclear metamorphosis from that which occurs in the better known ciliates like *Paramecium* or *Colpidium*, although in essence they are the same. In *Paramecium caudatum* four of the daughter micronuclei form four new macronuclei, losing their identity as micronuclei with the metamorphosis. In *Blepharisma* four, that is, all of the micronuclei, form macronuclei but retain their identity within the organs which they create, remaining and dividing within the macronuclear membrane until the next following conjugation.

Compared with the rhizopods the relations of idio- and trophochromatin are here reversed. In the former the idiochromatin is extruded from the trophochromatin or vegetative nucleus, remaining in the cell body as chromidia. In *Blepharisma* the trophochromatin is excreted from the micronucleus resulting in the 'purification' of the idiochromatin which remains permanently in the macronucleus as the micronucleus. In both cases it amounts to the same thing in the end, both idio- and trophochromatin coming from the syncaryon, or its immediate derivatives.

It is not improbable that other forms of ciliates in which micronuclei have not been observed in the vegetative stages, will be found to have nuclear relations analogous to those in *Blepharisma*. Here, also we have a clue to the explanation of the nuclei of forms like *Opalina* where dimorphic nuclei are absent. Such nuclei can be interpreted as amphinuclei in which idio- and trophochromatin are not separated but remain permanently as simple nuclei. In these the idiochromatin prevails over the trophochromatin in so far as the vital reproductive processes are concerned. In the majority of other ciliates the dimorphic conditions are fully established, an illuminating stage being seen in *Blepharisma undulans* where the two remain together, but, nevertheless, are differentiated. From such a condition to that in forms like *Paramecium* is

but a step in advance. The syncaryon of *Blepharisma* divides twice before nuclear differentiation. In *Paramecium* it divides three times. If the four nuclei of *Blepharisma* should divide again, and by the division should separate tropho- from idiochromatin, the former then swelling into macronuclei, the conditions would be identical with those of *Paramecium*.

Such an hypothesis of the origin of the dimorphic nuclei of ciliates is much more plausible than one based upon hypothetical binucleated ancestral forms. Metcalf ('09) for example, has advanced an elaborate theory requiring a number of suppositions: first, a delay in the process of division, establishing a temporary binucleated condition; second, a complete suppression of the delayed division, making the binucleated condition permanent; third, a shifting of division planes from longitudinal to transverse. Not one of these suppositions has sufficient evidence to warrant it. The facts indicate that the macronucleus represents the trophochromatin contained in the original syncaryon from which it is derived either by secretion, as in *Blepharisma*, or by differentiating division, as in *Paramecium* and its allies. No complicated phylogenetic hypothesis is needed in the light of these.

The second problem suggested by the history of *Blepharisma undulans* is the much discussed matter of conjugation and its significance. The process and its sequences in *Blepharisma* throw no new light on the subject but rather add to the perplexity of the tangle by an additional difficulty, for *conjugation in Blepharisma under cultural conditions, is equivalent to a death warrant*. Bütschli ('76) noted that ex-conjugants of *Blepharisma lateritia* invariably die: "Länger gelang es mir nun nicht die aus der Conjugation hervorgegangenen Thiere lebend zu erhalten: schon am zweiten Tage nach lösung der Syzygie starben viele ab, der Rest am dritten Tage" (p. 316). Nevertheless he made no remark on this apparent exception to his theory of Verjüngung in explaining the significance of conjugation. Maupas, likewise, observed fruitless conjugations, interpreting them as due to close relationship. A few other observers have also called attention to the invariable death of ex-conjugants. Baitsell ('11),

for example, isolated 132 pairs of closely related conjugating *Stylonychia pustulata*: "None of these ex-conjugants divided and none lived forty-eight hours after separating."

Two hundred pairs of conjugating *Blepharisma undulans* have been isolated and followed in culture after conjugation. The observations were made at different times during the year, to allow for possible seasonal predilection. The results have confirmed Bütschli's observations in every respect. Not a single ex-conjugant divided and the majority died within ten days; two, only, dragged out a miserable, weakened existence for twenty days. They become smaller and more vacuolated, until finally they bear little resemblance to the original robust forms from which they came (figs. 10, 11, 23).

If this negative result were common to all ciliates in culture there might be a more reasonable chance of interpreting conjugation. But this is not the case, nor is it common even amongst paedogamous ex-conjugants. *Paramecium*, *Colpidium*, *Gastrostyla*, *Euplotes*, *Vorticella*, *Didinium*, and a score of other ciliates have been cultivated after conjugation. *Stylonychia*, with *Maupus*, continued to live. Death after conjugation, therefore, seems to be rather an exception than a rule.

There is reason to believe that failure to live after conjugation is due to the conditions in the laboratory cultures. We have found, for example, that while ex-conjugants of *Paramecium caudatum*, from natural ponds or other external sources, continued to live in the proportion of from 70 to 80 per cent, those derived from prolonged cultures continued to live in the proportion of only 6 per cent (Calkins '03, Cull '07). The reason for this discrepancy must be sought in the conditions under which conjugation occurred. So, too, it is probable that the failure of *Blepharisma* to live after conjugation is due to some imperfection in the cultural conditions in the laboratory. Here the 'infant mortality' is 100 per cent, but in *Paramecium caudatum* under culture, the infant mortality was 94 per cent, almost as bad, while under conditions more nearly like those in nature the rate fell to 30 or 20 per cent. The theoretical interest centers in the 6 per cent of the culture *Paramecium*

that continued to live. Jennings, according to a recent paper ('12), might argue that, since these 94 per cent of ex-conjugants die, conjugation does not imply rejuvenescence as Bütschli, Maupas, and others have maintained. The continued life of the 6 per cent, on the other hand, indicates that these varied in some way from the others and represented a different mixture of germ plasms. If we go back to the original records of these 6 per cent we find that in no case, either exogamous or endogamous, did both individuals of the syzygy continue to live, one lived, the other died after from one to thirteen divisions. It is evident then, that even the admixture of the germ plasms from the same cells is not equally potent. The result may be interpreted either as a fortunate combination of pronuclei, or as a particularly favorable cytoplasmic medium in which the syncaryon acts, or both together. In any case it may be granted that conjugation does involve the possibility of increased variability, but this does not exclude the older view of rejuvenescence or renewal of vitality through conjugation. We have repeatedly shown that a change in the chemical composition of the medium will counteract a period of depression in organisms maintained on a constant diet, and renew the vitality, as indicated by the increased division rate. Engelmann ('76) early showed that conjugation results in the 'reorganization' of the cell, and it is now a well-known fact that in this process of reorganization the old macronucleus fragments and ultimately disappears in the cytoplasm. This disappearance must give rise to a great increase in the nucleo-protein content of the cell, therefore to a new chemical composition of the cell as a whole. We have recently shown that, under certain conditions, nucleo-proteids (especially the purines), have a markedly stimulating effect on the rate of cell division (Calkins, Bullock and Rothenburg '12). With this chemical change in the cell there is a condition which, theoretically, should work in the same general way as a chemical change in the surrounding medium and give rise to a renewal of vitality. The syncaryon in *Paramecium caudatum*, as we have seen, divides three times without cell division, and eight nuclei are

formed. Four of these nuclei become macronuclei, four micronuclei. These are formed during the process of disintegration and absorption of the old macronucleus. Then follows two divisions of the cell, resulting in four individuals, each with the normal nuclear relations. Each cell is now a new individual with renewed chemical basis and newly combined nuclei. The power to vary follows from the increased powers of metabolism, and it seems to us that the phenomena of conjugation are more reasonably interpreted as processes which have developed for rejuvenating effects whereby powers of metabolism, irritability, reproduction, and variation are reinvigorated, rather than as processes for engendering variations. If it is argued that 100 per cent of *Blepharisma* die after conjugation and, therefore, that rejuvenescence is not an effect of conjugation, the same argument may be returned and the question asked: Where does variation come in when 100 per cent die? We believe that unknown, probably cultural, conditions enter into the problem and make conjugation even more difficult to interpret under either hypothesis.

June, 1912.

LITERATURE CITED

- ANIGSTEIN, P. 1912 Beobachtungen über zwei neuen Ciliatenarten. Arch. f. Prot.
- BAITSELL, G. A. 1911 Conjugation of closely-related individuals of *Stylonychia*. Proc. Soc. Exp. Biol. Med., vol. 8, p. 74.
- BALBIANI, E. G. 1860 Recherches sur les phenomenes sexuelles des Infusories. Jour. de la Physiologie, tom. 4.
- BÜTSCHLI, O. 1876 Studien über d. erst. Entwicklungsvorgan. d. Eizelle. d. Zelltheilung u. die Conjugation der Infusorien. Abh. d. Senckenberg. naturforsch. Gesell. Frankfurt a/M., Bd. 10.
- CALKINS, G. N. 1903 Studies on the life history of Protozoa, I. Arch. f. Entwicklungsmechan., Bd. 15, p. 139.
- CALKINS, G. N., BULLOCK, F. D. AND ROTHENBERG, G. L. 1912 The effects of chemicals on the division rate of cells. Jour. Infect. Diseases, May.
- CULL, SARA W. 1907 Rejuvenescence as a result of conjugation. Jour. Exp. Zool., vol. 4.

- EHRENBERG, C. G. 1831 Ueber die Entwicklung und Lebensdauer d. Infusionsthier. Abh. d. Berlin. Akad., p. 1.
- ENGELMANN, W. 1876 Ueber Entwicklung und Fortpflanzung von Infusorien. Morph. Jahrb., Bd. 1, p. 573.
- JENNINGS, H. S. 1912 Harvey Lecture. Pop. Sci. Mo., May.
- LEBEDEW, W. 1908 Trachelocerca phoenicopterus. Arch. f. Prot., Bd. 13, p. 70.
- METCALF, M. M. 1909 Opalina. Arch. f. Prot., Bd. 13.
- MÜLLER, O. F. 1786 Animalcula Infusoria, etc. Leipzig.
- NERESHEIMER, C. 1907 Fortpflanzung eines parasitischen Infusors (Ichthyophthirius). Sitz. Ber. f. Morph. u. Physiol., München.
- PERTY, R. 1849 Mikroskopische Organismen der Alpen u. d. italienischen Schweiz. Mitt. Naturforsch. Ges. in Bern.
- 1852 Zur Kenntniss kleinster Lebensformen, etc. Bern.
- STEIN, F. 1867 Der Organismus der Infusionsthier. Bd. 2. Leipzig.

PLATE 1

EXPLANATION OF FIGURES

Same magnification throughout

- 1 Normal *Blepharisma undulans* with macronucleus in typical vegetative condition after division. From total preparation.
- 2 Giant specimen with macronucleus in division stage but with no indication of cell division. Three micronuclei shown. From total preparation.
- 3 Section showing details of peristomial region and two micronuclei.
- 4, 5, 6, 7 Successive stages in cell division. Note beginning and development of posterior adoral zone. From total preparations.
- 8 Normal nuclear relations in a dividing cell. Seven of the eight micronuclei shown within the macronuclear membrane. From section.
- 9 Involution truncated form after casting off the elongated posterior end. New vacuoles replace those lost. From total preparation.
- 10 Typical ex-conjugant. Two nuclei from the first division of the syncaryon on opposite sides of the old macronucleus. Note the small size of the cell as contrasted with figure 1. Total preparation from the right side.
- 11 Degenerating ex-conjugant five days after separation. From total preparation.
- 12 Conjugating *Blepharisma* with macronuclei, enigmatical micronucleus, and four micronuclei in the late anaphase of mitosis—evidently the second maturation division. From total preparation.



PLATE 2

EXPLANATION OF FIGURES

13 Interchange of micronuclei in form of minute spheres. (These may be fused nuclei and not pronuclei, in which case the two smaller nuclei are 'reduction nuclei.') From section, $\times 1000$.

14 Interchange of micronuclei. Four pronuclei in the region of interchange. Seven reduction nuclei in individual on right, four on left. From section, $\times 1000$.

15 Union of pronuclei. Three reduction nuclei in same vicinity in each cell. From section, $\times 1000$.

16 Conjugating individuals. The syncaryon in each is swollen and the macronuclei are undergoing fragmentation. From section, $\times 500$.

17 Early stage in conjugation, with the first changes of the micronuclei. Method of fusion and overlapping of the adoral zone distinctly shown. From total preparation, $\times 400$.

18 Syncaryon in each cell preparing for the first division. Breaking up of the macronucleus within its capsule. From section, $\times 1000$.

19 Full mitosis of the syncaryon, first division. From section, $\times 1000$.

20 Second division of the syncaryon (on left). A third enigmatical body in each cell. From section, $\times 1000$.

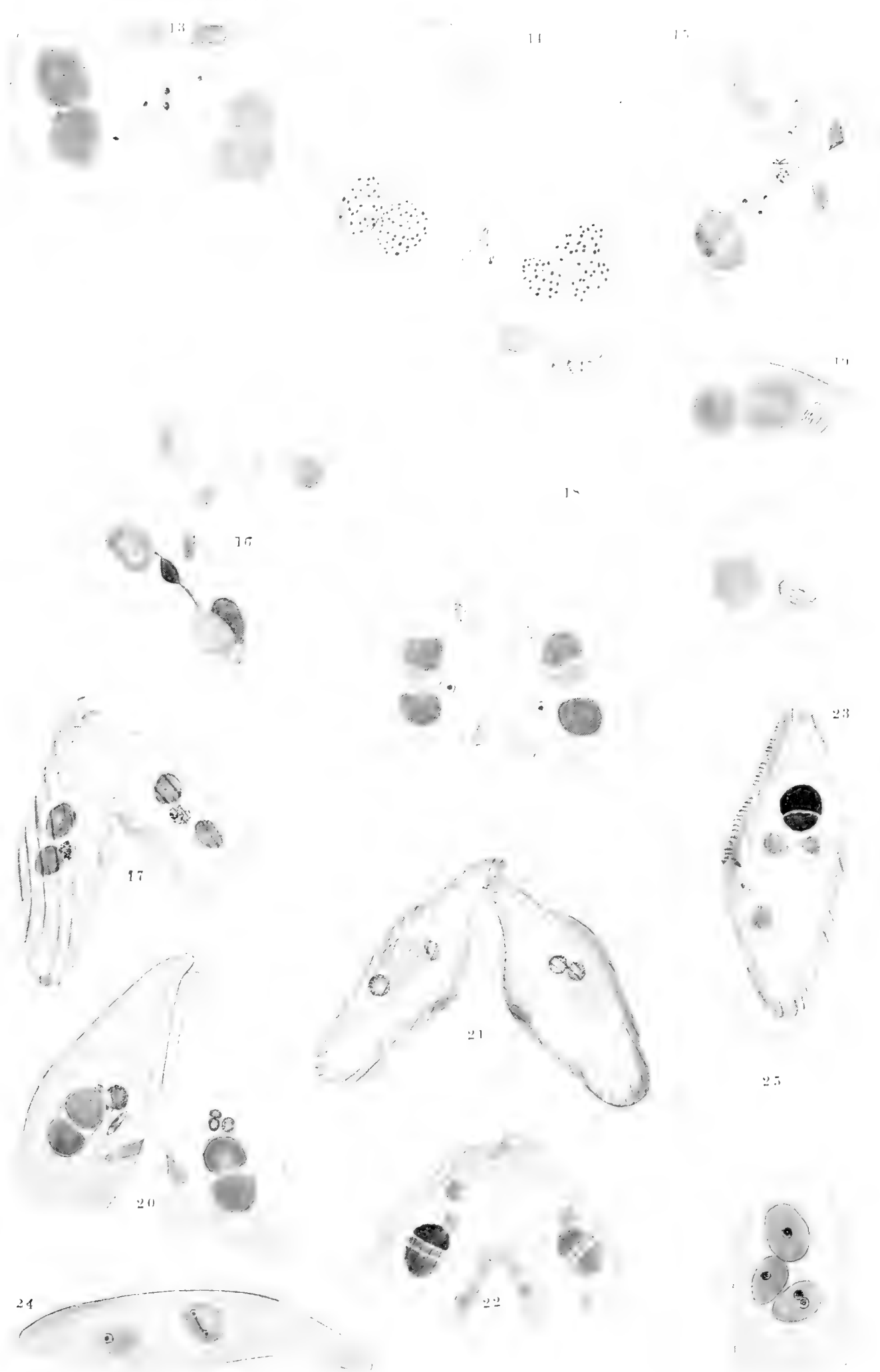
21 Pause after first division of the syncaryon. End stage in the separation of the conjugants. From total preparation, $\times 400$.

22 Vacuolization of the zone of fusion preparatory to separation. Nuclei in stage of pause. From total preparation, $\times 400$.

23 Ex-conjugant in same stage as that of figure 21. From total preparation, $\times 400$.

24 Ex-conjugant showing two new macronuclei with central micronuclei. The micronucleus in one has just divided as shown by the connecting strand. From section, $\times 500$.

25 Ex-conjugant showing three new macronuclei (the fourth is in another section). From section, $\times 500$.





THE DEVELOPMENT OF THE SKULL OF EMYS LUTARIA

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THIRTY-ONE FIGURES

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INTRODUCTION

Although certain parts of the chondrocranium of the chelonians have already been studied to some extent, the only previous work which has aimed to furnish a picture of the skull and its development in its entirety has been that of Parker ('80) on the development of the skull of *Chelone viridis*. His results are of great value, but on account of imperfect methods, many details of structure and development escaped him and several errors occur. A preliminary account of the present work has already appeared (Kunkel '11).

Of recent papers which deal with the chelonian chondrocranium especial reference should be made to that of Ogushi ('11) on *Trionyx japonicus* in which the cartilaginous as well as the bony elements of the adult skull are carefully described, and to the extensive paper of Nick ('12) on *Dermochelys coriacea*.¹ Neither of these deals with the embryonic condition of the skull. Several papers on the development of special regions may be referred to briefly. Seydel's ('96) conclusions regarding the development of the nasal capsules are confirmed, but several new facts which throw light on the significance of some of the characters of this region are set forth here for the first time. Noack's ('07) conclusion that the columella arises solely from the otic capsule is faulty in that my series shows the columella to be in reality made up of a stapes which arises external to the capsule and an extracolumella as has been described by Fuchs ('07) and Bender ('11). My own results accord with those of these investigators regarding the independence of columella and otic capsule. As to the significance of the stapes and extracolumella my results confirm Bender's as opposed to Fuchs's. The latter's conclusions regarding the development of the visceral skeleton I am able to confirm. Filatoff ('06) has called attention to the presence of a processus ascendens of the palatoquadratum in *Emys*.

Besides these papers may be mentioned also Filatoff's ('07) on the metamerism of the head of *Emys*, Fuchs's ('07) on the development of the roof of the mouth, and Thäter's ('10) on the same subject. Hitherto a complete account of the embryonic chondrocranium of the turtle and its development has been lacking so that comparisons have been possible only on the insecure basis of the adult condition.

In order to obtain a clear representation of the form relationships of the embryonic skull, a model was made by the Born wax

¹ In addition to these, should be mentioned a preliminary report by Fuchs (Ueber einige Ergebnisse meiner Untersuchungen über die Entwicklung des Kopfskelettes von *Chelone imbricata*. Verhandl. anat. Gesellsch., 26. Versamml. 1912, pp. 81-106.) Unfortunately further reference to it is impossible at this time. Of special importance is his demonstration of the derivation of the trochibasic skull from the platybasic type.

plate method of an embryo of *Emys lutaria* in which the carapace measured 11 mm. in length. The model represents the cartilaginous skull magnified fifty times with the membrane bones of the left side removed. At this stage the cartilage is well developed and ossification has not proceeded far enough to alter materially the original form of the chondrocranium. The membrane bones are fairly well developed although the parasphenoideum and quadratojugale as well as the complementare of the lower jaw appear first in older embryos than that modelled. The present study undertakes to determine the course of development of the chelonian skull from its early prechondral stage. A series of embryos and young with carapace lengths ranging from 4.7 mm. to 28 mm. forms the basis of the work.

It gives me pleasure to make the following acknowledgments: to Herr Professor Gaupp for the suggestion of the problem in the first place as well as for his constant valuable criticism and suggestions, and for the generous loan of many series of sections of *Lacerta* and *Chelone* embryos and of many papers from his private library; to Herr Professor Keibel who furnished me with a large number of embryos of *Emys lutaria* which were collected by Mehnert; and to Herr Geheime Rat Wiedersheim for the courtesies and facilities of the Anatomisches Institut of Freiburg i. B. where most of the work was done during the college year of 1910-11. To my father I am under obligation for material aid without which the present investigation could hardly have been undertaken.

GENERAL FORM OF THE SKULL

The cartilaginous skull of *Emys lutaria* resembles rather closely in its essential features that of *Lacerta* which has been so fully described by means of models by Gaupp ('00). The Chelonian skull is less elongated than that of *Lacerta*, especially in the anterior half, as is shown by the relatively anterior position of the fenestra hypophyseos. This is due principally to the fact that the septum interorbitale of the turtle is much shorter than in the lizards and the olfactory capsule, as will be shown later, is bent ventrally so that it comes to lie to a greater extent ventral rather than anterior to the orbital portion of the skull. Besides being

of more compact form on account of the shortening of the space between the olfactory and otic capsules, the numerous fenestrae of the lizard skull are reduced both in number and size. At the same time also, the slender rods of the lizard's skull are to a great extent replaced by broad, more or less continuous, plates. In this respect the chelonian chondrocranium resembles more nearly that of *Sphenodon* as described by Schauinsland ('00) and Howes and Swinnerton ('01) and that of the crocodile described by Parker ('83) than it does that of the lizards (Gaupp '00) and snakes (Parker '78). The greater strength of the jaws of the adult chelonian and crocodilian seems to be early foreshadowed in the embryo by the greater solidity of the skull, especially in the portions more intimately associated with the jaws. Notwithstanding, however, this greater solidity of the chelonian chondrocranium, in one respect it seems to be weaker in that it lacks the taenia marginalis which couples the orbital region with the otic capsule dorsally. In *Emys* the orbital and temporal regions are discontinuous except for the trabeculae which lie near the mid-ventral line, and the temporal and otic regions are discontinuous except ventrally because of the absence of the taenia marginalis. More detailed comparisons of the reptilian chondrocrania will be made below, under the respective parts of the skull.

PLANUM BASALE AND CHORDA DORSALIS

The basal plate forms the entire floor of the parachordal portion of the chondrocranium (fig. 25) extending forward from the occipital condyle through the occipital and otic regions. It is simple and continuous as far forward as the anterior half of the otic capsule where the hexagonal fenestra basicranialis posterior is situated. In front of this space the floor of the skull is represented by a heavy, transverse bar, the crista sellaris, which forms the hinder boundary of the fenestra hypophyseos (*f.h.*).

There can be but little doubt that the part of the skull here referred to as basal plate is made up in part, in the otic region, from the floor of the otic capsule. In a much younger stage (carapace length, 4.7 mm.) the boundary between the blastema of the basal plate and that of the otic capsule is quite distinct.

From this embryo it is seen that the blastema of the otic capsule extends medially slightly beyond the median wall of the membranous labyrinth, so that the basal plate proper is greatly reduced in width, and its lateral margins are slightly concave antero-posteriorly because of the position of the floor of the otic capsule. The fusion between the floor of the otic capsule and basal plate is complete in the stage modelled so that no line of demarcation is visible.

In general form the basal plate in the stage modelled may be regarded as hexagonal, exhibiting short anterior and posterior sides extending transversely, and antero-lateral and postero-lateral sides. Extending along the mid-ventral line from in front of the occipital condyle to the fenestra basiscranialis posterior is a low, rounded crest formed by the chorda dorsalis which in *Emys* lies in the same plane with and enclosed by the basal plate and at this stage is so large as to cause the latter to bulge both dorsally and ventrally in the regions where the basal plate is quite thin. Immediately behind the fenestra basiscranialis posterior, the basal plate on each side of the middle line exhibits a gentle convexity on its ventral side which is caused by the extension of the pars cochlearis as will be described later. The postero-lateral and antero-lateral margins of the basal plate do not pass directly over into the lateral portions of the occipital and otic regions, as in *Lacerta*, but show a tendency to project freely laterally in the form of crests. The postero-lateral margin projects laterally and slightly ventrally as the crista inferior (*c.i.*, fig. 1), whose posterior end terminates freely at the side of the base of the condylus occipitalis. This crest lies ventral to the anterior end of the fissura metotica and foramina spino-occipitalia which thereby come to open into a groove between the crista inferior and the lateral portion of the occipital region. This groove, lateral to which the ganglia vagi and spino-occipitalia lie, may be called the 'sulcus supracristularis' (*s.s.*, fig. 1). Laterally the margins of the basal plate project beyond the otic capsule, except in the posterior portion of the latter, to form a crista substapedialis (*c.st.*, fig. 4), a horizontal shelf which is continuous anteriorly with the crista basiptygoidea and on which the foot plate of

the columella auris rests ventrally, and under which the ramus communicans n. facialis cum glossopharyngeo lies. The anterolateral margin of the basal plate projects as a long ridge which passes directly into the trabecula cranii in front. This is the crista basipterygoidea (*pr.b.*, fig. 7) and represents the processus basipterygoideus of *Lacerta*, as will be shown later. It is inclined slightly ventrally so that a shallow groove is formed along the margin of the ventral surface of the basal plate in which lie the ramus palatinus n. facialis and arteria carotis interna (fig. 7). Accordingly it corresponds to the sulcus cavernosus of the adult pterygoideum (*s.c.*, fig. 25).

The dorsal surface of the basal plate exhibits a longitudinal ridge in the middle line which becomes less conspicuous posteriorly, disappearing completely in the condylar region. It is produced by the chorda dorsalis whose diameter is greater than the vertical thickness of the basal plate along the middle line. The thickness of the basal plate varies, decreasing markedly from the posterior end.

The fenestra basicranialis posterior (*f.b.p.*, fig. 25) is of a broad, hexagonal form with anterior and posterior margins extending transversely and with lateral angles. It lies in the anterior half of the basal plate between the anterior portions of the two otic capsules, with its anterior margin formed by the crista sellaris. At the stage modelled it is closed by a membrane of dense connective tissue beneath which the chorda dorsalis extends.

The crista sellaris (*c.s.*, fig. 25) forms a heavy transverse bar between the fenestra basicranialis posterior behind and the fenestra hypophyseos in front. In the mid-ventral line it exhibits a pronounced longitudinal ridge, separating two longitudinal grooves, while its lateral margin projects freely ventro-laterally continuous with the crista basipterygoidea. Within the groove thus formed, between the median crest of the crista sellaris and the projecting lateral crests, lie ramus palatinus n. facialis and arteria carotis interna.

Between the crista inferior and the pars lateralis of the occipital region extends the sulcus supracristularis, a wide groove facing laterally. In front it is limited by the posterior wall of the otic

capsule. Along the dorso-median wall of the sulcus open the three foramina spino-occipitalia and at its anterior end opens the ductus perilymphaticus. The ganglia vagi and spino-occipitalia are situated lateral to the groove.

The foramina spino-occipitalia and facialis lie rather in the lateral portion of the cranium than in the basal plate, but they may be conveniently described at this time. There is some variation in the number and relations of the foramina spino-occipitalia in the series of embryos studied, although they always lie in two horizontal lines, which converge in front, and are ventral to the bases of the arcus occipitales and fissura metotica. They pierce the basal plate in a ventro-lateral direction and open exteriorly in the sulcus supracristularis. In the individual modelled there are three pairs of foramina, of which the anterior is the smallest and the posterior the largest. The middle foramen is equally distant from the other two. In the adult, as is well known, the first and second pairs of nerves leave the cranial cavity through the same foramen, and only two pairs of foramina spino-occipitalia are present. In two younger embryos having a carapace length of from 7 to 8 mm. the adult condition was met with. In one embryo, older than that modelled, the first and second foramina were united at their external ends but completely separated from each other internally. On the other hand in one of the oldest individuals studied all three pairs of foramina were quite distinct as in the model.

The foramen facialis (*f.f.*, fig. 6) lies well in front of the anterior end of the otic capsule and relatively far laterally in the side portion of the otic region. Behind this foramen the basal plate is continuous with the otic capsule for a long space. In front of it there is only a slender commissure uniting the basal plate and the cupula anterior of the capsule.

The foramen abducentis (*f.a.*, figs. 8 and 24) passes horizontally forward at the anterior end of the crista sellaris, as a moderately long canal opening anteriorly ventral to the pila prootica and dorsal to the crista basipterygoidea, and posteriorly on the dorsal surface of the basal plate immediately behind the proximal end of the pila prootica.

The basal plate is separated from the cupula posterior of the otic capsule by the triangular, ventral portion of the fissura metotica; and from the cupula anterior by the fenestra prootica. Between these two foramina the basal plate is continuous with the capsule except for the foramen facialis which is so situated as to leave a slender commissure uniting the basal plate and otic capsule between itself and the fenestra prootica.

The relations of the chorda dorsalis to the basal plate are of considerable interest. In the embryo modelled the chorda passes without interruption from the anterior face of the dens epistrophei into the posterior end of the condylus in which region it is completely imbedded in cartilage. It is surrounded directly by a sheath which is continuous with the perichondrium of the basal plate in front and from which, in the region between the dens and condylus, the ligamentum apicis dentis is derived.

In front of the condyle, where the basal plate becomes gradually thinner, as far forward as the fenestra basicranialis posterior, the chorda lies in the same plane with the basal plate and tends to divide it into two symmetrical halves. The basal plate, accordingly, is parachordal, as has been already described in the skull of snakes, crocodilians, and *Sphenodon*; and not hypochordal as in *Lacerta*. This condition, however, is probably secondary, since in younger individuals in which the condyle is not yet developed, the basal plate immediately in front of the condylar region is hypochordal to within a short distance of the fenestra basicranialis posterior where it becomes parachordal.

The tissue immediately dorsal and ventral to the chorda in the region posterior to the fenestra basicranialis posterior is reduced to a very thin layer and is not fully chondrified in the stage modelled. In passing through the fenestra basicranialis posterior the chorda is enclosed in a membranous sheath which is continuous with that which fills the fenestra, although it comes to lie in a plane ventral to the basal plate.

The chorda dorsalis is lodged posteriorly on the dorsal surface of the crista sellaris in a deep groove whose sides gradually close together anteriorly, converting the groove into a canal which

extends forward through the crista and opens on its anterior surface into the fenestra hypophyseos.

In passing through the crista sellaris the chorda tapers from the uniform diameter which it exhibits posteriorly to a rounded point.

As might be expected in a part undergoing rapid degeneration, the anterior end of the chorda exhibits considerable variations in its relations to the crista sellaris. In several specimens it was bent in a dorso-ventral direction within the crista so that the anterior portion lay in a plane dorsal but parallel to the posterior portion. In most of the embryos studied the crista was completely perforated by the canal for the chorda, but in one young embryo the canal terminated within the cartilage. In another embryo only slightly younger than that modelled, the anterior end of the chorda projected freely into the fenestra hypophyseos while in the model, as in most of the specimens studied, the end of the chorda was flush with the anterior surface of the crista.

At the extreme caudal end of the condylus in the embryo modelled, the chorda is completely surrounded by cartilage, but in a very slightly younger embryo the condylus at its extreme caudal end exhibits a U-shape in cross section quite similar to that of *Lacerta*; that is, the chorda was surrounded only ventrally and on the two sides with cartilage (fig. 15). Later, however, the chorda is completely surrounded. Immediately surrounding the chorda the cartilage becomes excavated to form a cup-like depression, the central cavity, into which the dens epistrophei fits. The chorda passes from the anterior face of the epistropheus into the posterior face of the condyle, forming a condylus anularis characteristic of the *Chelonia*. The ventral surface of the condyle articulates with the atlas which projects ventrally to it.

In contrast with the condyle of *Lacerta*, as Gaupp has shown, that of *Emys* does not exhibit the two processes, one on each side of the chorda dorsalis, but rather a single ring-like process around the chorda dorsalis. In view, then, of the embryonic condition in *Lacerta*, the derivation of the mammalian condyles from those of reptiles is not improbable. In *Chelone*, according to Gaupp, the chorda enters the condyle from the ventral side so that,

even in the adult of this form, the central cavity of the condyle, in which the ligamentum apicis dentis is inserted, lies near the ventral margin of the posterior surface of the condyle.

REGIO OCCIPITALIS

The occipital region, like that of *Lacerta*, may be differentiated into a basal and two lateral parts. A dorsal region is lacking, although the tectum posterius, which is continuous with the otic capsules, and hence properly belongs to the otic region, projects caudally with its strongly developed processus posterior and closes in the foramen occipitale magnum dorsally.

The basal portion is represented by the basal plate including the condylus and cristae inferiores (fig. 1), and the lateral parts by the arcus occipitales, which are continuous ventrally with the basal plate and extend freely dorsally, separated from the otic capsules in front by the fissura metotica and not united distally with the tectum posterius. Because of the separation distally of the arcus occipitales from the tectum, it is evident that at this stage the fissura metotica and foramen occipitale magnum are not completely separated from one another.

The condylus occipitalis projects posteriorly as a cylindrical process with convex ventral surface and flat, or even slightly concave, dorsal surface so that a cross section is reniform. Its free distal surface is flat except for a slight depression which extends dorsally from the canal in which the chorda lies and gradually deepens toward the dorsal surface of the condyle. The free end of the condylus is embraced, except for a short space on the dorsal side, by the atlas, which has the form of an incomplete ring and projects forward ventral to the condyle as a stout process.

In later stages the ossification of the condylus is seen to proceed from three centers, one ventral to the chorda and one on each side of it; corresponding to the basioccipitale and two pleurooccipitalia respectively.

The lateral portions of the occipital region are represented by the arcus occipitales which arise from the dorsal surface of the basal plate in the region of the foramina spino-occipitalia. The arcus occipitales are stout, curved, slightly tapering prismatic

rods which recall strikingly the neural arches of the vertebrae. Their medial margins are curved regularly to enclose the foramen occipitale magnum laterally; their external margins, however, exhibit a more angular contour because of a marked thickening midway between their base and apex against which the otic capsule rests posteriorly. The fissura metotica is accordingly greatly narrowed here but not completely obliterated. The arcus occipitales bound the foramen occipitale magnum laterally and the fissura metotica posteriorly and medially.

The foramen occipitale magnum is large and of hexagonal form, with its dorsal and ventral margins transverse and with lateral angles. Its plane is vertical and transverse. The condyle and basal plate form its ventral margin, the curving arcus occipitales its lateral margins, and the free posterior margin of the processus posterior of the tectum posterius its dorsal margin.

REGIO OTICA

In contrast to the occipital the otic region is complicated. The basal plate forms the floor, the otic capsules the lateral walls and the tectum posterius the dorsal portion. Of the basal plate there should be mentioned the large, hexagonal fenestra basiscranialis posterior in the anterior part of the otic region and bounded anteriorly by the crista sellaris. The antero-lateral margins of the basal plate are extended ventro-laterally beyond the connection with the otic capsule, and form the posterior end of the crista pterygoidea. The lateral margin of the basal plate extends laterally, beyond the anterior and middle thirds of the capsule, as the crista substapedialis (*c.st.*, fig. 4). Posterior to the level of the fenestra vestibuli the lateral margin of the basal plate passes into the lateral capsular wall; in front of this fenestra, however, the lateral extension of the basal plate becomes gradually more pronounced.

The basal plate and otic capsule are in connection with each other for a considerable space, extending from the fenestra prootica in front to the foramen jugulare behind and interrupted only by the foramen facialis which is well in front of the cochlear portion of the otic capsule and separated from the fenestra pro-

otica by a short and comparatively slender rod, the commissura praefacialis which extends dorsally and laterally from the basal plate to the ventral aspect of the anterior cupula of the otic capsule. Behind the foramen facialis the median wall of the cochlea passes continuously into the basal plate along a curved line which is concave on the lateral side.

The prefacial commissure, together with the anterior cupula of the capsule, forms the posterior margin of the fenestra prootica in which is located, immediately below the capsule, the ganglion semilunare of the nervus trigeminus (*g.s.*, fig. 7). Unlike the condition in *Lacerta*, the ganglia of the three rami are closely united so that they appear as a single ganglion wholly within the fissure, except the anterior extremity which lies external and ventral to the pila prootica; whereas in *Lacerta* the ganglion of the ramus ophthalmicus lies widely separated from the others, quite far in front of the slender pila prootica.

The fissura metotica (*f.m.*) is a narrow slit, widening ventrally to a large triangular space, situated between the otic capsule on the one hand and the basal plate and arcus occipitalis on the other. The otic capsule forms the anterior and lateral boundary of the fissure, and the arcus occipitalis, the posterior and medial one. In its narrow dorsal portion the capsule comes to lie somewhat external to the occipital arch so that the fissure opens nearly transversely, while its extensive ventral portion faces laterally. Dorsally the fissure is continuous with the foramen occipitale magnum as already described, although its dorsal portion is closed by a mass of connective tissue.

In the expanded ventral portion of the fissure are situated the nervus vagus, vena jugularis, and ductus perilymphaticus in their passage to the exterior of the skull. The ductus perilymphaticus occupies the extreme ventral corner of the fissure. Dorsal to the ductus perilymphaticus, but still within the triangular expansion of the fissure at its ventral end, are situated the jugular vein and vagus nerve which in the adult pass out of the cranium through the foramen jugulare externum (*f.m.*, fig. 1).

Beneath the canalis perilymphaticus on the left side of figure 2 there may be seen a narrow groove which becomes wider in a pos-

terior direction from the section figured and which becomes converted into a very fine tube anteriorly by the growing together of the walls of the groove dorsally. In the embryo modelled the tube extends through only two or three sections and ends blindly posterior to the cavum cochleae. It apparently is the *canalis hypoperilymphaticus* which Nick ('12) has described in *Dermochelys*, *Chelydra*, and *Chelone*; like that of the two latter it contains no blood vessels but only a very loose connective tissue. In an older embryo (carapace length of 13.5 mm.) this canal has increased considerably in size so that it is one-third as large in diameter as the *canalis perilymphaticus* and it communicates anteriorly with the cavum cochleae at the most ventral and posterior portion of the latter. While the *canalis hypoperilymphaticus* apparently is not differentiated at a much earlier stage than that modelled, in an individual recently hatched of 28 mm. in carapace length, the canal was large.

The posterior wall of the cochlea, which is perforated by the large, oval *fenestra cochleae*, bounds the *fissura metotica* in front. This *fenestra* opens anteriorly into the cavum cochleae along the median wall of the capsule and posteriorly into the extreme anterior end of the *sulcus supracristularis*. The anterior end of the *sulcus supracristularis* corresponds quite closely with the *recessus scalae tympani*, as described by Gaupp in *Lacerta*. This region communicates with the otic capsule by means of the *fenestra cochleae*, with the cranial cavity by the extreme anterior end of the *fissura metotica*, and opens widely throughout its extent to the exterior of the cranium. These communications represent respectively the *fenestra cochleae* (*s. rotunda*), *apertura medialis recessus scalae tympani*, and *apertura lateralis recessus scalae tympani*.

The principal differences in the relationships of these three openings in *Lacerta* and *Emys* lie in the fact that the plane of the *fenestra cochleae* in the latter is vertical, while that of *Lacerta* is horizontal, and also in that the front margin of the *apertura lateralis recessus scalae tympani* lies slightly behind that of the *apertura medialis* in *Emys*. These slight differences are explicable by the fact that the cochlea in *Emys* has developed in a pos-

terior direction, as is also indicated by the relation of the nervus glossopharyngeus to the otic capsule. This change in relations may be imagined to have taken place by supposing that the posterior extension of the floor of the cochlea has occurred principally in front of the fenestra cochleae and has been more rapid on the antero-ventral aspect of the sac than on the postero-dorsal. In this event it is apparent that a foramen, for example, which originally lay in the floor of the capsule will be rotated to occupy the posterior wall. In the same way also, should the growth in a posterior direction be more rapid laterally than medially where the proximity to the basal plate would retard the extension, the external aperture of the recess would come to be somewhat posterior to the internal aperture.

CAPSULA OTICA

In general form the otic capsule may be compared to a triangular prism with one long edge situated ventrally and with the broadest face vertical and medial; accordingly there are an anterior and a posterior base and also a latero-dorsal and latero-ventral face, as well as a median one. The two prisms lie with their axes horizontal and their median faces diverging only slightly from ventral to dorsal and from posterior to anterior. In contrast to the condition in *Lacerta* the lateral walls of the cranium are more fully represented by the otic capsules and the brain is confined more completely by them without exhibiting a tendency to extend laterally dorsal to the capsules. On account of the unusual extension of the sacculus and lagena in a ventral direction the height of the capsule is almost as great as the greatest length of the same. The two bases of the prismatic capsule project in its long axis as a cupula anterior and posterior, the latter of which is the more pronounced. The cupula anterior bounds the dorsal portion of the fenestra prootica posteriorly and projects freely without connection with other parts of the skull. The cupula posterior bounds the fissura metotica anteriorly and laterally, extending caudally to lie external to the occipital arch as already described. The two capsules are united dorsally by the tectum posterius whose slender lateral rods arise

gradually from them as triangular plates. The comparatively simple prismatic form is greatly modified by a number of prominences which for the most part follow the underlying parts of the membranous labyrinth though not as closely as in *Lacerta* because of the greater thickness of the capsular walls.

The capsule may be differentiated into a dorsally situated vestibular portion and a smaller, ventrally situated cochlear portion which encroaches upon the basal plate, as already described. The vestibular portion exhibits prominences corresponding to the semicircular canals, with their ampullae, and the utriculus; the cochlear portion which remains simpler in its external form, exhibits a flattened, oval, pocket-like form.

The *prominentia semicircularis anterior* (fig. 28) forms the dorsal and antero-dorsal margins of the otic capsule. Its ventral end, situated in the cupula anterior, widens to accommodate the ampulla anterior which lies in a somewhat oblique position so that it does not produce a marked convexity in the capsular wall. The plane of the anterior semicircular canal inclines medially from ventral to dorsal and from anterior to posterior. The *prominentia semicircularis posterior* is continuous in front with the anterior prominence and curves along the dorsal and posterior margins of the capsule to the cupula posterior. The dorso-median edge of this prominence is continuous for its middle third with the *tectum posterius*. The plane of the canal inclines medially from below and in front, so that its ventral end projects laterally much as does that of the anterior canal. The *prominentia ampullaris posterior* forms a marked convexity on the lateral wall of the capsule below the ventral end of the posterior semicircular canal and dorsal to the *crista parotica* (*cr.p.*, fig. 28); the *prominentia ampullaris posterior* accordingly forms the postero-ventral margin of the capsule and bounds the anterior end of the *fissura metotica*. On the median wall it forms a continuous area with the gently bulging *prominentia utricularis* which forms an area continuous in front with the *prominentia sinus superioris utriculi*, ventrally with that of the *sacculus*, posteriorly with the *prominentia ampullaris posterior*, and dorsally with the *prominentia semicircularis posterior*. On the median

capsular wall the prominences of the anterior and posterior semicircular canals unite with the dorsal end of the *prominentia sinus superioris utriculi*.

The *prominentia semicircularis lateralis* with that of its ampulla forms a horizontal ridge which is situated on the lateral surface of the capsule between the ventral ends of the anterior and posterior canals, and which broadens anteriorly to accommodate the ampulla. A decided triangular depression marks this ridge off dorsally from the other two canals. Ventrally it passes gradually into the *prominentia saccularis* except at its anterior end where the *prominentia recessus utriculi* is situated.

The *prominentia recessus utriculi* on the lateral aspect of the otic capsule occupies a circular space ventral to the combined prominences of the ampullae lateralis and anterior, dorsal to the foramen facialis, and antero-dorsal to the fenestra vestibuli. On the median face of the capsule it forms a gentle triangular convexity with its posterior angle situated immediately dorsal to the foramen acusticum posterius; its antero-ventral angle dorsal to the foramen facialis and anterior to the foramen acusticum anterius. The *prominentia recessus utriculi* is separated above from that of the anterior semicircular canal by the shallow fossa subarcuata; it passes imperceptibly into the *prominentia ampullaris anterior* in front, and is bounded ventrally by a line joining the foramina facialis and acusticum posterius; it is continued posteriorly between the latter and the foramen endolymphaticum and forms a marked convexity for the ductus endolymphaticus immediately dorsal to its opening into the sacculus, and ventral to the foramen endolymphaticum.

The *prominentia sinus superioris utriculi* extends obliquely antero-dorsally from the foramen endolymphaticum to unite with the dorsal ends of the prominences of the two vertical semicircular canals. In front it forms the posterior boundary of the fossa subarcuata, posteriorly it is continuous with the combined *prominentiae utricularis* and *ampullaris posterior*.

The *prominentia saccularis* occupies the entire ventral third of the capsule. In contrast to the condition met with in most of the vertebrates, the cochlea of the chelonians extends posteriorly

instead of anteriorly so that the unique condition is met with in that the nervus glossopharyngeus passes through the cavity of the cochlea in its passage from the cranium to the exterior, as will be described more fully later. On its lateral aspect the *prominentia saccularis* is semicircular with its convexity below. It is situated behind the foramen facialis and the *prominentia recessus utriculi* and beneath the *prominentia semicircularis lateralis*, and passes posteriorly beyond the foramen glossopharyngei externum. Its ventral wall is the basal plate. As already mentioned the *prominentia saccularis* is apparent from the ventral side of the skull as a gentle convexity of the basal plate which lies behind the level of the fenestra basicranialis posterior. On the median aspect of the otic capsule the prominence extends posteriorly from the region of the foramen acusticum anterius posteriorly as far as the anterior angle of the fissura metotica. The convexity of the *prominentia saccularis* medially is more pronounced in its anterior portion immediately behind the slender side piece through which the n. facialis passes than posteriorly so that the foramen acusticum anterius, which is situated in this region, comes to face somewhat anteriorly as well as medially.

The crista parotica forms a stout prismatic structure with its ventral surface horizontal and extending forward from the ventral limits of the *prominentia ampullaris posterior* and projecting freely for a very short distance in front.

The fenestra vestibuli (s. ovalis) (*f.v.*, fig. 28) is situated in the ventral part of the lateral wall of the otic capsule, in the middle of the saccular region between the foramen glossopharyngei externum and foramen facialis, slightly nearer the former than the latter. It is of equilateral triangular form to accommodate the foot plate of the columella auris. Its ventral margin is horizontal and is formed by the basal plate which projects laterally beyond the capsule as a kind of horizontal shelf on which the foot plate of the columella rests (fig. 5).

Behind the fenestra vestibuli and separated from it by a slender rod sloping obliquely postero-ventrally, is the external opening for the n. glossopharyngeus (*f.g.e.*, fig. 28). This is

situated ventral to the *prominentia ampullaris posterior* and at the postero-dorsal angle of the *prominentia saccularis*.

On the median wall of the otic capsule are five or six foramina arranged in two horizontal rows. In the ventral row are the foramina *acusticum anterius* and *posterius* and the median opening for the glossopharyngeal nerve. The foramen *acusticum anterius* is small and is situated above and behind the foramen *facialis* and ventral to the *prominentia recessus utriculi*. Close behind the anterior foramen is the much larger, oval foramen *acusticum posterius* which is situated in the antero-dorsal portion of the *prominentia saccularis*. Posterior to and separated by a considerable space from the last is the small foramen *glossopharyngei internum*. It lies close in front of the *fissura metotica* and at the upper margin of the *prominentia saccularis* and below the *prominentia ampullaris posterior*. In the upper row of foramina are the foramen *endolymphaticum*, a foramen for a small blood vessel and a third foramen which may or may not be present and which has apparently no significance, being simply an unchondrified area. The foramen *endolymphaticum* is situated postero-dorsally from the foramen *acusticum posterius* and in front of the broad *prominentia sinus superioris utriculi*. The small foramen for a blood vessel lies in front of the foramen *endolymphaticum*; and on the left side only in the individual modelled between these two foramina is the insignificant unchondrified area already mentioned.

Viewed from the lateral side the otic capsule (fig. 28) exhibits the following topography. The anterior margin of the cupula anterior projects slightly laterally as a rounded ridge, the *prominentia semicircularis anterior*. Below the ventral end of this, in the space above the foramen *facialis*, is a circular convexity, the *prominentia recessus utriculi*, which extends behind as far as the *fenestra vestibuli*. Extending horizontally from the ventral end of the *prominentia semicircularis anterior* to the ventral end of the *prominentia semicircularis posterior* is the conspicuous cylindrical ridge comprising the *prominentiae ampullaris lateralis* and *semicircularis lateralis*; ventrally this prominence merges without a sharp boundary into the *prominentia saccularis*,

dorsally it is separated from the prominences of the vertical semicircular canals by a deep depression. The *prominentia saccularis* is interrupted by the large *fenestra vestibuli* as well as by the *foramen glossopharyngei externum* at its extreme upper and posterior angle. The *prominentia semicircularis posterior* rounds off the capsule postero-dorsally and rests ventrally on the *prominentia ampullaris posterior* which is separated by a slight depression from that of the semicircular canal.

The median aspect of the capsule has a much more uniform surface than the lateral one. The prominences of the two vertical semicircular canals make a less conspicuous ridge framing the capsule above than they do on the lateral side. Ventral to the *prominentia semicircularis anterior* is the conspicuous depression, the *fossa subarcuata*, which separates it from the *prominentia recessus utriculi*. Posterior to the ventral end of the *prominentia semicircularis anterior* and ventral to the *fossa subarcuata* is the inconspicuous triangular *prominentia recessus utriculi* which is dorsal to the *foramen facialis* and antero-dorsal from the *foramen acusticum anterius*. Its posterior angle extends dorsal to the *foramen acusticum posterius* and ventral to the *foramen endolymphaticum*. Posterior to the *foramen endolymphaticum* the *prominentia sinus superioris utriculi* extends diagonally forward and upward and continues at its upper end with the prominences of the two vertical semicircular canals; posteriorly it merges imperceptibly into the *prominentia utricularis* and *ampullaris posterior*. Ventral to the line connecting the dorsal margins of the two *foramina acustica* and *glossopharyngei internum* is the semicircular *prominentia saccularis* which bulges suddenly immediately behind the *foramen facialis* and extends backward to the *fissura metotica*.

The posterior wall of the cochlea is perforated near its median margin by the *fenestra cochleae*, the anterior end of the *canalis perilymphaticus*, as already described. The relation of these parts is complicated in the stage modelled on account of the extension posteriorly of the lateral wall of the cochlea so that the *canalis perilymphaticus* opens posteriorly into a funnel-like extension of the anterior end of the *sulcus supracristularis*.

In older embryos, as already described, in addition to the *canalis perilymphaticus* is also the *canalis hypoperilymphaticus* opening into the cochlear cavity from the *sulcus supracristularis*.

INTERIOR OF CAPSULA OTICA

The interior of the otic capsule (figs. 29 and 31) exhibits a complicated form, but in contrast to that of *Lacerta* it is somewhat simpler because of the absence of a *septum intervestibulare* and the consequent differentiation of *cava vestibuli anterius* and *posterius*. Besides this, the posterior development of the lagena tends to make the interior more compact. It is possible to distinguish a *cavum vestibuli* with a *cavum ampullare posterius*, *laterale*, and *anterius*, the three *canales semicirculares*, and a *cavum cochleae* which communicates widely with the *cavum vestibuli*. Of especial interest in comparison with *Lacerta*, in addition to the absence of a *septum intervestibulare* and the relation of the *cavum cochleae*, may be noted the *sulcus* in the posterior wall of the cochlea in which the *n. glossopharyngeus* is situated in its passage from the interior of the cranium to the exterior. It is slightly oblique in its course, extending slightly ventrally from the median to the lateral side on the posterior side of the *cavum cochleae* immediately below the *recessus ampullaris posterior*.

The principal part of the total cavity of the capsule is represented by that of the vestibule. Medially the *cavum vestibuli* extends to the median wall of the capsule, but the lateral wall of the capsule forms only a part of its lateral boundary, the vertically situated *septum* between the vestibulum and the *canalis semicircularis lateralis* (*septum semicirculare laterale*) being interposed in the space immediately in front of the *recessus ampullaris posterior* and behind that of the lateral ampulla (*s.s.l.*, fig. 4). The roof of the *cavum vestibuli* is incomplete because of the large *foramen pro sinu superiore utriculi*. The roof is formed by the *septa semicircularia anterius* and *posterius* which extend horizontally outwardly from the median wall of the otic capsule and separate the *canalis semicircularis anterior* and *posterius* respectively from the *cavum vestibuli*. The *septum semi-*

circulare posterius extends laterally in a horizontal direction to the septum semicirculare laterale, the septum semicirculare anterius to the lateral wall of the capsule. The floor of the cavum vestibuli is very limited in extent because of the wide connection ventrally between it and the cavum cochleae which lies below it. The whole cavity of the capsule including that of the lateral semicircular canal is somewhat flattened from side to side.

The cavum vestibuli may be differentiated for clearness in description into a central portion with posterior, vertical, and anterior limbs. The posterior limb is situated ventral to the septum semicirculare posterius and encloses the ampulla posterior and the median limb of the canalis semicircularis lateralis as well as the posterior portion of the utriculus (fig. 3, left side). The vertical limb is situated between the anterior end of the septum semicirculare posterius and the posterior end of the septum semicirculare anterius, and encloses the sinus superior utriculi (fig. 4). The anterior limb is situated below the septum semicirculare anterius, and encloses the ampullae anterior and lateralis and the recessus utriculi (fig. 6, left side). The central portion of the cavity is situated medial to the septum semicirculare laterale and encloses the principal portion of the utriculus and the recessus utriculi.

The posterior limb of the cavum vestibuli is pear-shaped in a vertical cross section, the narrow end being directed laterally and dorsally to accommodate the posterior semicircular canal, while the thickened end is medial and ventral for the accommodation of the posterior ampulla. Posterior to the septum semicirculare posterius, the orificium inferius canalis semicircularis posterioris opens ventrally into the cavum vestibuli on its dorsal aspect. Immediately ventral to this septum the canalis semicircularis lateralis opens in a vertical longitudinal plane into the lateral aspect of the posterior limb of the cavum vestibuli. Dorsally the cavum vestibuli communicates with the two vertical semicircular canals by means of a common orifice situated between the septa semicircularia anterius and posterius and near the median side of the cavity. Opening into the anterior limb of the cavum vestibuli in a vertical plane facing obliquely forward and laterally

is the *orificium inferius canalis semicircularis anterioris*. Behind this orifice and below the *septum semicirculare anterius* are the cavities of the lateral and anterior ampullae which are in the form of lateral pouches from the *cavum vestibuli*, that of the lateral ampulla being somewhat dorsal and posterior to that of the anterior ampulla. Ventral to the *cava ampullaria anterius* and *lateralis* is that of the *recessus utriculi* which forms a concavity in the lateral wall of the *cavum vestibuli*, so that at this point the *cavum vestibuli* exhibits its maximum width from side to side (fig. 6). The *cavum vestibuli* has a small bulging on its median side about midway between its posterior and anterior ends and at a level with the ventral end of the *sinus superior utriculi* for the accommodation of the *ductus endolymphaticus* as it proceeds ventrally from its foramen to its opening into the *sacculus* (fig. 4).

The *cavum ampullare posterius*, occupying the postero-ventral corner of the *cavum vestibuli*, is differentiated from the dorsally situated *canalis semicircularis lateralis* by a medial thickening of the lateral capsular wall on the one hand and a ventro-lateral ridge on the *septum semicirculare posterius* on the other (fig. 2); anteriorly the cavity of the posterior ampulla opens into the *cavum vestibuli*; and dorsally, into the posterior semicircular canal.

The *cavum ampullare laterale* lies lateral to the anterior end of the *cavum vestibuli* and opens medially into it by a wide mouth, anteriorly it opens into the *cavum ampullare anterius* and ventrally into that of the *recessus utriculi*; posteriorly it communicates with the *canalis semicircularis lateralis* through the wide *orificium anterius canalis semicircularis lateralis*. Its antero-dorsal boundary is formed by the *septum semicirculare anterius*, its lateral wall is that of the capsule itself; and medially it opens by a very wide aperture into the *cavum recessus utriculi* and the *sinus superior utriculi*. At its extreme dorsal end the *cavum ampullare laterale* is bounded medially by the posterior end of the *septum semicirculare anterius*.

The *cavum ampullare anterius* forms a kind of antero-lateral pocket of the *cavum vestibuli*, being continuous with the anterior

end of the *cavum recessus utriculi*. The *canalis semicircularis anterior* opens into it from above by the *orificium inferius canalis semicircularis anterioris*. The *cavum ampullare anterius* is differentiated from that of the *recessus utriculi* which lies behind it by a broad vertical ridge on the lateral capsular wall.

The *cavum utriculi*, which occupies the middle portion of the *cavum vestibuli*, is continuous dorsally with that of the *sinus superior utriculi*, anteriorly with the *recessus utriculi* and *ampulla lateralis*, posteriorly with the *ampulla posterior* and *canalis semicircularis lateralis*, and ventrally with the *cavum sacculi*. Its median wall is formed throughout by the median capsular wall; laterally it is bounded by the *septum semicirculare laterale* and, ventral to the septum, by the lateral wall of the capsule which is continuous with it. Anterior to the septum the cavity opens laterally into the lateral ampulla and the *recessus utriculi*, and posterior to the septum it is continuous with the median limb of the lateral semicircular canal. At its anterior end the *cavum utriculi* widens abruptly in a lateral direction to form the *cavum recessus utriculi*, which lies rather ventral to the lateral ampulla.

The *cavum sinus superioris utriculi* extends dorsally from the *cavum utriculi* between the *septa semicircularia anterius* and *posterius*. Opening into it from the anterior and lateral aspect are the *cava recessus utriculi* and *ampullare laterale*. Dorsally it opens into the *orificia superiora canalis semicircularis anterioris* and *posterius*. Medially and laterally it is limited by the respective capsular walls.

The *cavum recessus utriculi* forms a pocket extending ventrally from the *cavum ampullare laterale* and anterior to the *cavum utriculi*. Anteriorly it opens into the *cavum ampullare anterius* and ventrally into that of the *sacculus*, but at its extreme anterior end the ventral capsular wall limits it.

The *canalis semicircularis posterior* curves laterally and ventrally from its union with the *sinus superior utriculi*, in a posterior direction to the posterior ampulla, into which it opens from above by means of the *orificium inferius canalis semicircularis posterioris*. The *canalis semicircularis lateralis* connects with the posterior portion of the posterior semicircular canal by means of

the *orificium posterius canalis semicircularis lateralis* which is situated immediately ventral to the *septum semicirculare posterius* and somewhat lateral to the posterior semicircular canal.

The *canalis semicircularis anterior* curves from its posterior end, first in an antero-lateral direction, then ventrally, and at its extreme antero-ventral portion, somewhat medially, so that its entrance into the *cavum ampullare anterius* through the *orificium inferius canalis semicircularis anterioris* is from above and laterally. It is bounded dorsally, medially, anteriorly, and in part laterally by the corresponding walls of the capsule; ventrally it is bounded by the *septum semicirculare anterius* which also lies somewhat lateral to the canal at its posterior end. The antero-lateral end of the canal is limited behind by a medial thickening of the lateral capsular wall which separates the cavities of the anterior and lateral ampullae.

The *canalis semicircularis lateralis* has two quite distinct portions, a median limb and a lateral one. The cavity of the median limb is confluent with that of the utriculus, the posterior ampulla and the inferior end of the posterior canal. It lies lateral and dorsal to the utriculus and posterior ampulla and anterior to the posterior semicircular canal. The cavity of the median limb is bounded laterally by the *septum semicirculare laterale* and dorsally by the *septum semicirculare posterius*. The lateral limb is separated, except at its posterior end, from the median limb by the *septum semicirculare laterale*. The canal is horizontal in position and is continuous in front with the lateral ampulla through the large *orificium anterius canalis semicircularis*. Posteriorly the lateral limb of the lateral semicircular canal is continuous with the median limb immediately behind the *septum semicirculare laterale* and also with the ventral and posterior end of the posterior semicircular canal.

The *cavum sacculi* (*c.sac.*, fig. 29) exhibits several features of great morphological interest on account of the relations of the *ductus perilymphaticus* and the development of the *ductus cochlearis* in a posterior direction. It has the general form of a flattened pocket which is rather shallow in front and increases quite regularly in depth posteriorly so that the posterior wall is

high and vertical in position. Dorsally the cavum sacculi stands in wide open connection with that of the vestibulum. Its boundaries are the capsular walls laterally, medially, posteriorly, and anteriorly; and ventrally the basal plate which bulges ventrally as already noted because of the great extension of the lagena in a ventral direction. In its ventral portion the cavum sacculi exhibits an antero-lateral and a postero-medial lobe which stand in wide open communication with each other; the boundary between the two extending from the median wall at the level of the foramen acusticum posterius in a postero-lateral direction. The antero-lateral lobe extends on the outer aspect posteriorly beyond the fenestra vestibuli which interrupts its lateral wall; on its median aspect it extends posteriorly only as far as the foramen acusticum posterius. In it is situated the sacculus. The postero-medial lobe is somewhat deeper than the previous one so that its ventral end projects slightly below the level of the lateral lobe. It is somewhat triangular in form with the posterior side in a vertical plane. This encloses the lagena, the ductus perilymphaticus and the n. glossopharyngeus in its course between the external and internal foramina of that nerve. Accordingly this cavity exhibits two small but very important extensions; namely, the sulcus glossopharyngeus (*s.g.*) and the canalis perilymphaticus (*c.per.*). The sulcus glossopharyngeus is situated on the posterior cochlear wall ventral to the cavum ampullare posterius and extends transversely from the foramen glossopharyngei internum in a lateral and ventral direction to the foramen glossopharyngei externum. It is in the form of a cylindrical groove of uniform size which opens into the cavum sacculi as already indicated. Near its median extremity it intersects the ventral wall of the cavum ampullare posterius so that it shows communication with this part as well. It is completely filled by the nerve which thus comes to lie with its anterior half projecting freely into the cavum sacculi. The canalis perilymphaticus extends in an anterior direction from the fenestra cochleae horizontally through the posterior cochlear wall. It opens into the sacculus at a point half way between the dorsal and ventral ends of that part and somewhat medial to the middle plane of the cavity.

The canal opens at its anterior end in an oblique plane along the median wall of the cochlea a short distance in front of its extreme posterior end (fig. 3, left side). The ductus perilymphaticus fills the canal completely.

The septum semicirculare posterius is in the form of a horizontal plate which extends laterally from the median to the lateral capsular wall and septum semicirculare laterale. It passes dorsal to the cavum ampullare posterius and ventral to the canalis semicircularis posterior as has been already noted. The septum begins at its posterior end as a ridge which extends laterally from the median capsular wall and differentiates the canalis semicircularis posterior from its ampulla. It terminates abruptly anteriorly behind the sinus superior utriculi. Dorsal to the septum is situated the canalis semicircularis posterior while ventral to it are the ampulla posterior, the utriculus, and the median limb of the lateral semicircular canal. The two latter are differentiated from each other by a broad ridge on the under side of the septum.

The septum semicirculare laterale is a vertical plate situated in a longitudinal plane separating the ampulla posterior, the cavum vestibuli, and median limb of the lateral semicircular canal on the one hand from the lateral limb and ampulla on the outer side. At its posterior end the lateral margin of the septum semicirculare posterius and the lateral capsular wall unite.

The septum semicirculare anterius is a plate which extends between the lateral and median capsular walls in an oblique position, its anterior end lying somewhat higher than its posterior. It lies dorsal and medial to the ampullae lateralis and anterior, and ventro-lateral as well as posterior to the canalis semicircularis anterior. The septum is slightly twisted; at its posterior end its lateral margin lies at a higher level than its median margin, while at its anterior end the lateral margin is lower than the median margin. In front the septum arises from the median capsular wall as a ridge which projects laterally and differentiates the anterior semicircular canal from its ampulla. Posteriorly the septum arises from the lateral capsular wall as a ridge which demarcates the cavum sinus superioris utriculi from that of the lateral ampulla.

TECTUM POSTERIUS

The tectum posterius, which is the only part of the chondrocranium roofing in the central nervous system dorsally, is continuous ventro-laterally with the otic capsules, and so may properly be described with the otic region notwithstanding the fact that it extends posteriorly to close in the foramen occipitale magnum above. The tectum posterius may be differentiated into a median and two lateral portions. The latter are slender cylindrical rods which slant medially and dorsally from the upper margin of the otic capsule in order to support with their distal median ends the large median portion which is of rhomboidal form with its long axis in the sagittal plane. This median portion accordingly exhibits a long, slender triangular processus ascendens which slants upward in an anterior direction and a stouter processus posterior which is more nearly semicircular in form and extends with its postero-ventral extremity to limit the foramen occipitale magnum above. In cross section the processus ascendens becomes crescentic toward its apex, with the convexity directed dorsally, while that of the processus posterior is more nearly oval. The tectum is slightly curved in the sagittal plane, thus exhibiting a postero-dorsal convexity and an antero-ventral concavity. The saccus endolymphaticus is situated close below and in front of the slender lateral portion.

CRISTA PAROTICA

The crista parotica (*cr.p.*, fig. 28) forms a short, longitudinal crest, extending in an anterior direction from the ventro-lateral aspect of the prominentia ampullaris posterior slightly beyond the foramen glossopharyngei externum, dorsal to which it ends freely in a vertical, transverse face (*cr.p.*, fig. 2). It is triangular in cross section exhibiting a ventral and a median surface, the latter attached to the otic capsule except at its extreme anterior end which projects freely. There is accordingly a dorso-lateral face which makes a broad, shallow groove with the part of the external wall of the otic capsule lying dorsal to it in which the postero-ventral portion of the quadratum rests.

Ventral to the crest runs the vena capitis lateralis which joins the v. jugularis interna immediately posterior to the crista parotica a short distance anterior to the fenestra metotica and continues obliquely upward and forward over the lateral wall of the capsule ventral to the prominentia semicircularis lateralis.

A separate processus paroticus articulating with the crista parotica, and which Gaupp has described in *Lacerta*, is not present at any stage of *Emys* in the series studied.

RELATION OF THE NERVES IN THE REGIO OTICA

The relations of the nerves in the otic region of the skull exhibit several points of considerable interest. As described by Bojanus the n. glossopharyngeus passes directly through the cavity of the osseous labyrinth of the ear in *Emys*. In the stage modelled both the median and lateral walls of the cochlea are perforated near their posterior ends and immediately ventral to the vestibular portion of the capsule by the foramina glossopharyngei internum and externum respectively. The n. glossopharyngeus passes from the inner foramen to the outer one in a ventro-lateral direction. Between the two foramina the posterior wall of the cochlea is excavated, as has been already described, to form a deep groove, the sulcus glossopharyngeus, in which the nerve lies with its anterior surface freely exposed in the cochlear cavity. The dorsal wall of the groove is incomplete at its median end because of the intersection of the cavum vestibuli with the sulcus glossopharyngeus, so that the nerve has its dorsal as well as its anterior surface for a short space exposed freely in the cavity of the capsule.

With reference to the nervus glossopharyngeus, the lagena extends much further ventrally and posteriorly than it does in *Lacerta* in which form the n. glossopharyngeus leaves the skull together with the vagus through the fissura metotica which extends quite far forwards ventral to the pars cochlearis.

From the foramen glossopharyngei externum the nerve extends in a ventro-lateral direction to the ganglion petrosum (s. glossopharyngei) which lies ventral to the external fenestra and lateral to the sulcus supracristularis.

The nervus acusticus exhibits a ramus vestibularis and a ramus cochlearis. The former is the smaller of the two and is situated slightly dorsal and somewhat anterior to the latter. They enter the capsule through the foramina acustica anterius and posterius respectively. The anterior foramen is situated at the extreme anterior end of the pars cochlearis immediately ventral to the recessus utriculi; the posterior foramen is situated nearer the middle of the pars cochlearis, immediately in front of the postero-medial lobe of the cochlear cavity and ventral to the cavum utriculi.

The nervus facialis finds its exit from the cranial cavity through the foramen facialis which is situated a short distance dorsal to the basal plate and in front of the anterior wall of the cochlea. The ganglion facialis (s. geniculi) lies external to the chondrocranium, separated from it by a thin layer of connective tissue, in front of the foramen and dorsal to the lateral extension of the basal plate already mentioned. The ganglion, accordingly, rests in a broad groove between the basal plate on the ventral side and the prefacial commissure, which unites the basal plate and otic capsule, on the dorsal side. This groove is apparently the homolog of the fovea genicularis of the rabbit, as described by Voit ('08, p. 448).

The ramus palatinus nervi facialis arises from the lateral aspect of the ganglion facialis and passes in a ventro-medial direction around the free edge of the crista substapedialis and then turns in an anterior direction in the sulcus palatinus which is medial to the rudimentary processus basipterygoideus on the ventral side of the basal plate.

The ramus hyomandibularis arises from the posterior end of the ganglion geniculi and runs posteriorly in the fovea genicularis and comes to lie further laterally and dorsally as it proceeds and passes dorsal to the columella auris. At the level of the latter the ramus hyomandibularis gives off the chorda tympani which extends in a lateral direction along the dorsal margin of the columella and then turns sharply forward and ventrally to reach the mandible, as Noack ('06) has already described. The main

portion of the hyomandibular branch continues in a posterior direction in the space between the quadratum and the otic capsule.

At the point at which the palatine branch reaches the sulcus palatinus there is given off in a posterior direction the ramus communicans n. facialis cum n. glossopharyngeo which extends postero-laterally along the ventral surface of the chondrocranium beneath the pars cochlearis of the otic capsule, the crista substapedialis, and the planum basale to unite finally with the anterior end of the ganglion glossopharyngei.

REGIO ORBITO-TEMPORALIS

The orbito-temporal region shows the same general configuration as that of *Lacerta* except that it is of much heavier and more compact structure, being made up of broader plates instead of slender rods. The fenestrae are also less numerous and relatively smaller than in *Lacerta*, in which respect the chelonian skull resembles more closely the primitive condition of *Sphenodon*. The whole region has the form of a shallow longitudinal trough, within which the anterior end of the brain is supported on its ventral side. Ventral to the trough, the septum interorbitale (*s.i.*, fig. 9) lies in a sagittal plane and increases in height from the posterior to the anterior end. The sides of the trough are formed by the pila prootica, the subiculum infundibuli, the pila metoptica, and the planum suprasedale. These walls are perforated by the fenestrae prootica, metoptica, optica, and foramen ophthalmicum, besides a small perforation in the subiculum infundibuli, present only on the right side of the embryo modelled which represents the beginning of the absorption of the cartilage at this point.

As in all the Sauropsida, the orbito-temporal region is differentiated into a posterior temporal and an anterior orbital portion. The temporal portion is continuous behind with the otic region and exhibits a basal and two lateral parts. The orbital region is characterized by the high septum interorbitale which forms a continuous wall between the two orbits. This septum is a thin vertical plate, as will be described later; in the stage modelled it is thin, but in younger embryos it is relatively thicker and made

up of two parallel plates which are united to each other only along the ventral margin by a high commissure (figs. 22 and 23), a condition which apparently may be referred to a primitive form in which the skull was platybasic. Anteriorly the septum interorbitale passes without interruption into the septum nasi, which in my youngest stages is apparently of unpaired origin.

The temporal region is made up of the trabeculae and pilae prooticae, both arising from the anterior aspect of the crista sellaris, the latter from the dorsal side, the former from the ventral side. The bases of these two structures are separated from each other by the nervus abducens which, as already mentioned, penetrates the crista sellaris ventral to the base of the pila prootica (VI, fig. 8).

The trabeculae project horizontally forward in the plane of the basal plate as triangular rods which converge in the median line and enclose the semicircular fenestra hypophyseos in front and on the two sides. They continue further forward as the thickened ventral margin of the septum interorbitale and fuse so that no trace of the paired nature of the septum remains. Posteriorly the trabeculae are continuous with the antero-lateral margins of the basal plate which represent the crista basipterygoidea, as already described.

At the level of the posterior margin of the fenestra hypophyseos a separate cartilago articularis, described by Gaupp in *Lacerta*, is represented by a rudimentary, imperfectly chondrified mass of tissue which is attached to the ventral edge of the crista basipterygoidea (*c.a.*, fig. 8). This cartilage forms a small, roundish knob, about two-thirds as broad as long, which projects downward from the crest with its free end directed anteriorly and ventrally. It extends lateral to the ramus palatinus n. facialis and its free end is embraced laterally as well as ventrally by the pterygoideum. In older embryos the crista basipterygoidea becomes relatively more prominent and is partly surrounded by the dorso-median portion of the pterygoideum, and the cartilago articularis disappears as a distinct piece, probably fusing with the crest (*c.pt.*, fig. 20).

The pila prootica which forms the lateral portion of the temporal region may be described in general as a broad U-shaped plate of nearly uniform width, with its convex side anterior and its concave side posterior, and exhibiting accordingly a median and a lateral limb. It is attached to the basal plate by the median limb, while the external limb of the U is somewhat dorsal to the former and ends freely close to and medial to the anterior cupula of the otic capsule. The plane of the whole plate is so curved that while it is nearly horizontal in a transverse direction at its attachment to the basal plate, the free lateral limb is nearly vertical in its position. The median limb is inclined antero-dorsally from its proximal end where it is attached to the front surface of the crista sellaris. The lateral limb of the pila exhibits a small, anteriorly directed process which approaches a posteriorly directed process from the postero-lateral angle of the planum supraseptale. These two processes narrow the fenestra metoptica dorso-laterally without closing it in completely, and suggest, in their position, an incomplete taenia marginalis. In another embryo, slightly younger than that modelled, this anteriorly directed process of the lateral limb is greatly enlarged and is perforated by a large foramen, the significance of which is still undetermined.

In the embryo modelled, the pila prootica grows very thin toward its dorso-lateral margin and passes gradually into a thin layer of dense connective tissue which extends dorsally and medially and encloses the cranial cavity above.

A peculiarity of the individual modelled, which has not been met with in any of the other specimens studied, is the presence of a short cylindrical rod-like process from the dorsal surface of the medial limb of the pila prootica near its distal end which extends postero-laterally in a horizontal plane and ends freely in the cranial cavity medial to the anterior margin of the fenestra prootica. The nervus oculomotorius, as it passes anteriorly over the medio-dorsal surface of the pila prootica in order to leave the cranial cavity through the fenestra metoptica, lies dorsal and medial to this process, while the n. trochlearis, which is approximately parallel to the oculomotor, lies lateral and dorsal to this same process. Beneath the pila prootica, running horizontally

forward from the anterior apex of the ganglion semilunare, are the nervi nasalis and frontalis of the ramus ophthalmicus.

The pila prootica bounds the fenestra prootica in front and on its two sides and forms the posterior margin of the fenestra metoptica. Immediately external to the fenestra prootica, and occluding it almost completely, is the ganglion semilunare whose anterior apex lies in front of the fenestra.

The fenestra hypophyseos is semicircular, the transverse front margin of the crista sellaris forming its posterior boundary and the two trabeculae enclosing it laterally and in front.

The arteria carotis interna enters the cranial cavity through the postero-lateral angles of the fenestra hypophyseos which are not excavated to form incisurae caroticae as in *Lacerta*. The ventral side of the crista sellaris, however, as already noted, bears a broad longitudinal groove on each side of the middle line, the sulcus palatinus, in which the arteria carotis interna, together with the ramus palatinus n. facialis, lies; so that the extreme anterior end of the sulcus where the artery turns dorsally to enter the fenestra hypophyseos might be looked upon as an indication of an incisura carotica. Posteriorly the sulcus palatinus continues as the sulcus cavernosus.

The anterior three-fourths of the fenestra hypophyseos is completely occupied by the hypophysis cerebri which has a slightly oblique position so that its anterior surface rests partly upon the subiculum infundibuli in front of the fenestra.

The subiculum infundibuli arises in front of the fenestra hypophyseos from the dorsal surface of the trabeculae as a thin plate made up evidently of two symmetrical halves which are slightly inclined toward each other in the middle line to form a shallow longitudinal trough. The infundibulum and antero-dorsal end of the hypophysis are supported by this plate. The subiculum infundibuli is triangular, with its apex directed posteriorly and its base anteriorly forming the hinder margin of the fenestra optica, and, with its antero-lateral angles prolonged to form the short pilae metopticae, and with its postero-lateral margins bounding the ventral half of the fenestra metoptica in front. The whole subiculum infundibuli inclines rather sharply upward in front,

being supported ventrally by the septum interorbitale which increases rapidly in height from its posterior end. The lateral margin as it passes over into the pila metoptica is incised by the broad sinus oculomotorius for the oculomotor nerve as it passes obliquely to the outside of the cranium through the fenestra metoptica. Near the anterior margin, the subiculum infundibuli is perforated by a pair of large oval foramina (foramen ophthalmicum, *f.o.*, fig. 25) through which the arteria ophthalmica passes to reach the orbit. In the embryo modelled there is a small fenestra on the right side only which represents the first step in the resorption of this part of the chondrocranium. The subiculum infundibuli is much thicker anteriorly and laterally than medially and behind, and in older embryos in which the resorption has been carried farther, the entire posterior part of the subiculum has disappeared and the foramen ophthalmicum becomes a sinus, opening freely behind into the fenestra metoptica. In older embryos the interorbital septum becomes fenestrated by a long, narrow opening which extends both anterior and posterior to the subiculum infundibuli, its anterior end lying beneath the fenestra optica, so that, as in *Lacerta*, a cartilago hypochiasmatica is differentiated.

The pila metoptica is a short, stout rod extending antero-laterally from the antero-lateral angles of the subiculum infundibuli to the posterior margin of the planum supraseptale. The nervus oculomotorius passes ventral to the pila metoptica, leaving the cranial cavity through the sulcus oculomotorius. The nervus trochlearis passes out of the cranial cavity through the fenestra metoptica lateral and dorsal to the nervus oculomotorius, lying parallel to the latter beneath the pila metoptica.

The fenestra prootica is a large, oval opening between the anterior cupula of the otic capsule and the prefacial commissure behind and the pila prootica in front. On account of the curved form of the latter the fenestra is enclosed by it dorsally as well; the closure, however, is not complete on account of the absence of a taenia marginalis and the failure of the pila prootica to fuse with the otic capsule, a condition which resembles *Sphenodon*. The fenestra is completely filled by the large ganglion semilunare

which extends with its anterior apex ventral to the pila prootica (*g.s.*, fig. 8). In EmtS the ganglia of the three branches of the n. trigeminus are consolidated into one mass unlike the condition in Lacerta where that of the ophthalmic branch is separated from that of the other two and lies quite far anteriorly.

The fenestra metoptica, through which the nn. oculomotorius and trochlearis leave the cranial cavity, is a narrow slitlike opening, having an oblique position, sloping forward and dorsally from its base. It is bounded posteriorly by the pila prootica, and anteriorly by the subiculum infundibuli, pila metoptica, and planum supraseptale. From its anterior side the fenestra is narrowed by the prolonged postero-lateral angle of the planum supraseptale and again by a short posteriorly directed process midway between this process and the lateral end of the pila metoptica. On the right side of the embryo modelled this second process is reduced in the median part of its length so that only a proximal stump remains projecting from the posterior margin of the planum supraseptale and a small isolated rod of cartilage which lies freely within the fenestra (fig. 24). In another embryo of approximately the same age, the fenestra metoptica becomes regularly wider laterally and is narrowed only by the single posteriorly directed process of the postero-lateral angle of the planum supraseptale. The fenestra metoptica is not fully closed dorsally because of the absence of a complete taenia marginalis, although, as already described, the projection from the front margin of the pila prootica and that from the hind margin of the planum supraseptale suggest together an incomplete taenia marginalis.

The fenestra optica (*f.opt.*, fig. 24) is of irregular triangular form, somewhat broader than long. The planes of the two foramina are slightly inclined toward each other in accordance with the obtuse angle made by the two halves of the planum supraseptale. The two foramina are separated from each other in the middle line only by the free dorsal margin of the septum interorbitale. Posteriorly they are bounded by the subiculum infundibuli, and for a very short distance postero-laterally, by the pila metoptica; anteriorly they are bounded by the planum supra-

septale. The orbital portion of the orbito-temporal region is characterized by the septum interorbitale which increases in height from behind and carries the planum supraseptale with its dorsal margin so that the floor of the cranium is raised.

The septum interorbitale is a triangular plate lying in the sagittal plane, arising posteriorly from in front of the fenestra hypophyseos and rapidly increasing in height anteriorly almost to its extreme anterior end where the dorsal margin inclines rapidly ventrally to pass into the septum nasi. The ventral margin is thickened throughout its length to a cylindrical rod which is continuous behind with the trabeculae and tapers anteriorly in the region of the olfactory capsule to a thin edge. Its dorsal margin is continuous with the subiculum infundibuli and planum supraseptale except in the region of the fenestra optica where it is free. The septum forms a continuous plate throughout with no fenestrae such as *Lacerta* exhibits, although at a later stage, as already mentioned, a long slit in the posterior portion of the septum ventral to the fenestra optica causes a cartilago hypochiasmatica to be differentiated.

Of especial interest in the stage modelled is the structure of the extreme anterior dorsal corner of the septum where it passes over into the planum supraseptale posteriorly and the commissurae speno-ethmoidales anteriorly. At this point the septum is very clearly paired, the right and left halves being separated from each other by a shallow groove which is continuous postero-dorsally with the troughlike planum supraseptale. By a comparison with younger stages the significance of this peculiarity becomes clear. In an embryo having a carapace length of 7 mm. the portion of the septum lying in front of the fenestra optica is represented by two vertical plates, parallel to each other which are fused together along their ventral edges (fig. 23). Dorsally these plates diverge rapidly from each other along a line corresponding approximately to the dorsal margin of the septum in the older embryo. In the region of the fenestra optica, and for a short distance in front of the fenestra, the septum is thick so that its ventral cylindrical margin is not differentiated from the rest

of the septum. In this young stage, accordingly, the cavum cranii extends ventrally between the two plates of the septum in the region between the eyes as a deep, narrow cleft.

The planum suprasedale forms a broad shallow trough in which the cerebral hemispheres and olfactory lobes rest. Its plane rises obliquely from behind, on account of the increasing height of the septum interorbitale, but at its extreme anterior end its plane falls rapidly ventrally so that it is almost vertical, a condition associated with the rotation ventralwards of the olfactory capsule as will be described later under the ethmoidal region. In general, it is broadly oval in form, somewhat wider than long, with its anterior and lateral margins evenly rounded, but its posterior margin irregularly indented as has been described above. Medially its posterior margin forms the front boundary of the fenestrae opticae and further laterally the front boundary of the fenestra metoptica; between these two fenestrae is the pila metoptica. The postero-lateral angle of the planum suprasedale is produced behind as a slender rod-like process which approaches the front edge of the pila prooptica. The postero-lateral portion of the planum suprasedale, corresponding somewhat in position to that of the fenestra epioptica of *Lacerta*, is perforated by numerous small foramina which represent the first stages in the absorption of the cartilage in this part of the cranium.

At its anterior end, the planum suprasedale becomes very narrow and passes into the anterior end of the septum interorbitale. At the same time the two halves of the planum suprasedale, which form an obtuse angle with each other throughout most of their length, come to lie at the extreme anterior portion with their median surfaces almost parallel and separated from each other by a narrow cleft.

The commissurae speno-ethmoidales, diverging from each other, extend in an antero-ventral direction from the extreme anterior margin of the planum suprasedale and serve to connect the walls of the olfactory capsule with the orbital region. They enclose the fenestrae olfactoriae laterally.

REGIO ETHMOIDALIS

The ethmoidal region of *Emys* exhibits greater differences from that of *Lacerta* than does any other region of the chondrocranium. It has been carefully described by means of models by Seydel ('96), whose results are in the main confirmed by the present study, and by Nick ('12) in *Chelydra serpentina*, *Chelone midas*, and *Dermochelys coriacea*. The homologies of certain parts, however, which were not discussed by Seydel, have been determined by study of a more complete series of embryos. To Seydel's observations there are only a few details to be added.

In striking contrast to the condition in *Lacerta*, the capsule is more compact and is made up of continuous plates of cartilage interrupted only to a limited extent by fenestrae and not modified by alar processes and a complicated concha; besides this, the whole capsule has undergone a bending in a ventral direction. The differences in general form of this region in *Lacerta* and *Emys* are to be correlated with the greater strength of the jaws of chelonians, which condition requires that the bones against which the lower jaw impinges (premaxillare, maxillare, vomer, palatinum) have a more solid foundation to rest against than in such forms as the snakes and lizards. Gaupp ('06, p. 45) has already called attention to the fact that the form of the olfactory capsule is controlled as well by the structure of the jaws as by the form of the olfactory sac itself.

The olfactory capsule is divided into two symmetrical halves by the septum nasi which continues in an anterior direction from the septum interorbitale. The postero-dorsal wall of the capsule accordingly forms the anterior boundary of the orbit and the anterior wall forms the anterior limit of the head. On account of its position relatively far ventral to the other portions of the skull, its ventral wall projects below the level of the lower edge of the septum interorbitale to afford a prominent, and at the same time solid, support for the upper jaw. Besides the septum nasi which forms the median wall of each half, the cartilages making up the walls of the capsule may be differentiated into the tectum nasi dorsally, the paries nasi laterally, the solum nasi and

the cartilago paraseptalis ventrally, and the planum antorbitale forming the postero-dorsal wall which separates the olfactory sac from the orbit. These regions for the most part pass over into each other by even transitions.

The entire dorsal and posterior portion of the capsule, as Seydel has already shown, is free from the septum as is also the case in *Lacerta*; that is, the planum antorbitale terminates freely above and behind. Besides the connection between the septum nasi and the walls of the capsule there is a further connection of the capsule with the rest of the skull by means of the commissura spheno-ethmoidalis, a cartilaginous rod extending posteriorly from the tectum nasi on each side to the anterior extremity of the planum suprasedale, where the latter passes into the septum suprasedale, and enclosing the two fenestrae olfactoriae on the sides, and thus separating the fenestra olfactoria from the fissura orbitonasalis.

The capsule opens in front to accommodate the apertura nasalis externa, by the fenestra narina, and posteriorly by the fenestrae basales for the choanae. There is a longitudinal slit in the floor of the capsule which Seydel has called the foramen praepalatium. The capsule is perforated dorsally by the fenestrae olfactoriae, ventral to which on each side is the fissura orbitonasalis, through the most dorsal part of which the ethmoidal branch of the ramus ophthalmicus n. trigemini passes in its course from the orbit to the olfactory sac. Besides these openings there is also a small foramen epiphaniale for the passage of the n. lateralis nasi rami ophthalmici from the capsule to the exterior. A foramen apicale is lacking in all stages studied.

In contrast to *Lacerta* the entire capsule is shifted ventrally and so rotated that the anterior end is slightly ventral to the posterior end. This change in position is indicated partly by the fact that the plane of the fenestra olfactoria is inclined from the horizontal, as in *Lacerta*, so that its ventral end is below the posterior. Besides this, the capsule is somewhat compressed laterally so that its floor is pressed ventrally and extends well below the lower margin of the septum nasi. The two halves of the capsule are thus separated on the under side by a groove which

becomes shallower in front; and the fenestra basalis is brought well below the ventral margin of the septum, the upper side of the fenestra standing at the level of the ventral margin of the septum (figs. 9 and 27).

The contour of the walls of the capsule follows closely that of the underlying olfactory sac. Accordingly the capsule may be differentiated into two distinct regions, a relatively high, somewhat conical pars olfactoria situated dorsally, and a broader cylindrical pars respiratoria, opening in front by the fenestra narina and behind by the fenestra basalis. These two regions are separated by a shallow groove which extends diagonally posteriorly and ventrally and becomes more pronounced at its posterior end. The depression causes the interior wall of the capsule to be thrown up into a corresponding ridge which supports the lateral 'Grenzfalte' of Seydel.

Above the groove just described is a second depression in the lateral wall of the capsule. It is somewhat shallower than the first and appears only along the middle third of the capsule. It is the external manifestation of a corresponding ridge in the interior of the capsule. The accompanying prominent fold of the nasal epithelium supported by the thickening of the lateral wall, is evidently a rudimentary concha. It extends posteriorly as far as the recessus ducti naso-pharyngei, which is immediately ventral to it. In older embryos than that modelled, the concha becomes much more prominent on the inner side of the paries nasi (fig. 19).

The planum antorbitale is a thin curved plate making up the posterior and postero-dorsal wall of the capsule. It slopes rapidly ventrally from its antero-dorsal end and passes with an even curvature laterally and anteriorly into the paries nasi. In the stage modelled it is separated medially from the septum nasi by the fissura orbitonasalis so that it has a free margin which is rolled inwardly to project into the cavity of the capsule, parallel to the septum (fig. 12). The infolded margin is broadest in front; behind it disappears entirely in the region of the fenestra basalis.

The tectum nasi is short in an antero-posterior direction and comparatively narrow from side to side. In front it falls sud-

denly ventrally to form the front wall of the capsule dorsal to the fenestra narina. Posteriorly it passes into the planum antorbitale and is continuous with the septum nasi and commissura spheno-ethmoidalis. Between the septum nasi and the anterior extremities of the commissurae spheno-ethmoidales accordingly, the tectum bounds the fenestra olfactoria in front. Laterally the tectum nasi bends smoothly ventrally to form the paries nasi. Anteriorly it slopes abruptly ventrally and almost vertically to enclose the pars olfactoria in front. On account of the rounded form of this part, the tectum nasi exhibits in this region two gentle convexities separated from each other by a groove marking the position of the front margin of the septum nasi with which it is in perfect continuity. Below each of these cupulas, immediately above the fenestra narina, there is a short blunt, anteriorly directed process which, in later stages, becomes more conspicuous. To this process may be given the name *processus supranarinus* (*pr.s.*, fig. 25).

The paries nasi forms a practically continuous plate of cartilage of slightly convex form limiting the olfactory capsule laterally. It is continuous medially and dorsally with the tectum nasi as well as with the planum antorbitale; ventrally throughout its entire length it is continuous with the solum nasi. Its anterior end passes into the tectum nasi dorsally and ends freely ventrally as the lateral margin of the fenestra narina. This free margin is incised to form a very broad shallow sinus (*sinus externus*) for the accommodation of the duct of the glandula nasi externa which empties into the entrance passage of the olfactory sac (figs. 14 and 27). Posteriorly the paries nasi continues with the solum nasi to form the cartilago ectochoanalis (*c.e.*, fig. 9) which bounds the fenestra basalis ventro-laterally. Above the cartilago ectochoanalis the posterior margin is deeply incised to form a rounded sinus in which the recessus ducti nasopharyngei lies and which I would call the sinus nasalis posterior. The paries may be differentiated into a dorsally situated pars olfactoria and a ventrally situated pars respiratoria corresponding to the respective parts of the olfactory sac lying within. These two regions are separated from each other by a broad, shallow groove, which extends

horizontally backwards from the level of the upper margin of the fenestra narina to the apex of the sinus nasalis posterior and at the same time grows shallower. The paries nasi is of uniform thickness throughout except in the region immediately posterior to the groove on the exterior, which marks the position of the concha. Here the wall of the capsule is thickened to form a more prominent, rounded ridge on the inner surface. In front of this thickening the paries nasi exhibits on its medial surface a ridge (corresponding to the groove already mentioned on the external surface) which supports a fold of the epithelium of the olfactory sac representing a rudimentary concha (fig. 11).

The floor of the nasal capsule offers the most striking differences from the conditions met with in other reptiles. The solum nasi projects in front ventral to the fenestra narina to form the processus infranarinus, which thus limits the sinus externus below and medially. Behind the fenestra narina the solum nasi is continuous with the septum for a short space, and laterally bends upward to unite with the paries nasi with an even curvature. This portion of the solum nasi represents the lamina transversalis anterior of Lacerta. Posterior to this portion the solum nasi is separated from the septum by an elongated slit to which Seydel has given the name foramen praepalatinum (*f.p.* fig. 25). Behind this foramen the floor of the capsule again unites with the septum. In this region the ventral margin of the septum inclines strongly dorsally in a posterior direction. From the posterior margin of the solum there projects posteriorly a short cartilago paraseptalis which bounds the fenestra basalis medio-ventrally and is separated from the septum by a narrow sinus paraseptalis. Laterally the solum nasi at its transition to the paries nasi is prolonged, as already mentioned, to form a short spout-like process extending for a short distance posteriorly and supporting the ductus naso-pharyngeus ventrally and laterally, which may accordingly be called the cartilago ectochoanalis. The lateral margin of this limits the sinus posterior below.

The significance of that portion of the solum nasi which is continuous with the septum between the foramen praepalatinum in front and the fenestra basalis behind is difficult to understand.

In a younger embryo than that modelled (fig. 30) the solum nasi is separated from the septum completely behind a slender lamina transversalis anterior so that the entire posterior half of the solum nasi is free from the septum much as in *Lacerta*, as Gaupp has shown. Accordingly the foramen praepalatinum of *Emys* corresponds to the anterior portion of the cleft separating the septum from the cartilago paraseptalis, and the portion of the solum nasi which is continuous with the septum behind the foramen praepalatinum represents a portion of the cartilago paraseptalis which has become extended medially and fused with the septum. The solum nasi exhibits in the region lateral to the cartilago paraseptalis in the younger embryos several foramina which in the embryo modelled have become occluded but which are still manifest as thinner spots in the floor of the capsule.

The cartilago paraseptalis of *Emys* is relatively much shorter than that of *Lacerta* and does not unite in the stage modelled with the planum antorbitale by its posterior end. At a later stage there is such a fusion similar to that in *Lacerta*. But, unlike the condition in *Lacerta*, the choanae of *Emys* lie well posterior to the cartilago paraseptalis instead of anterior to the posterior end of the cartilage.

The relationship of parts of the floor of the capsule of *Emys* may be derived from that of *Lacerta* by a widening in a median direction of the cartilago ectochoanalis so that the fenestra basalis is moved posteriorly by an obliteration from in front, and at the same time by a broadening of the cartilago paraseptalis in its posterior half so that it fuses with the septum. That these changes may actually have taken place is indicated by the nasal capsule of the younger embryos in which, as has already been noted, the cartilago paraseptalis is separated from the septum for its entire length, and by the fact that, lateral to the cartilago paraseptalis, the floor of the capsule is perforated by a series of fenestrae arranged in a longitudinal row.

Anteriorly, the solum nasi projects forward as a short process ventral to the apertura nasalis externa; it passes over smoothly into the paries nasi on the two sides. While the floor of the capsule is nearly horizontal, except for a ventral crest along the lower

margin of the septum, which Parker ('80) termed the 'prenasal cartilage,' farther posteriorly the capsules extend much farther ventrally so that there is a fairly deep median groove between the two bulging capsules. The solum nasi projects posteriorly as the cartilago ectochoanalis. Throughout the greater part of its length the solum nasi is continuous with the septum, the foramen praepalatinum and the slit separating the cartilago paraseptalis and septum being the only points at which the floor is incomplete.

The septum interorbitale continues into the septum nasi interrupted only by a small circular perforation situated near the dorsal margin immediately in front of and between the bases of the commissurae spheeno-ethmoidales, so that there is formed a very high ventral commissure and a slender, rodlike one between the septum interorbitale and septum nasi.

The septum nasi is a thin vertical plate with a longitudinal ridge on each side, the crista longitudinalis septi, which extends from in front at the level of the upper margin of the fenestra narina posteriorly and ventrally to the point at which the cartilago paraseptalis becomes separate from the septum (figs. 13 and 18). The septum is much shorter along its dorsal than along its ventral margin, and is continuous anteriorly with the tectum nasi, but posteriorly, in the region of the fenestra olfactoria, it ends freely dorsally. The upper margin slopes downward in front. The ventral margin is continuous with the floor of the capsule except in the region of the foramen praepalatinum and that of the short cartilago paraseptalis. Like the upper margin it slopes downward in front, but more rapidly than the upper margin so that the septum nasi is much higher in front than behind. Especially in the region of the anterior half of the foramen praepalatinum the septum projects below the level of the floor of the capsule to form a slight ventral crest along the mid-ventral line, the prenasal cartilage of Parker. The front margin of the septum is continuous in its dorsal half with the transverse vertical front wall of the capsule and rounds off gradually into the dorsal margin; in its ventral half the septum ends freely between the two fenestrae narinae to separate them completely from each other. In a somewhat older stage of *Emys* than that modelled, Seydel

describes the septum as not extending as far forward as I have found it, so that the two fenestrae narinae are less perfectly separated from each other.

Of special interest in connection with the septum nasi is a long rodlike cartilage which, at its posterior end, is continuous with the septum by the crista longitudinalis septi; it extends anteriorly, parallel to the crest and separated from it by a narrow space, and ends freely a short distance behind the anterior margin of the septum. The glandula nasalis media is medial and ventral to this rod while lateral to it and supported by it is the median fold of the olfactory sac which separates the pars olfactoria from the pars respiratoria. To this rod I would give the name pila supraglandularis (*p.sg.*, fig. 13).

In an embryo of 13.5 mm. carapace length the pila supraglandularis is continuous at its posterior end with the floor of the capsule immediately in front of the foramen praepalatinum (figs. 16 to 18). From this relation it follows, as Seydel has shown, that the foramen praepalatinum opens into the olfactory capsule directly in a dorsal direction behind and in a lateral direction further forward beneath the pila supraglandularis. Later in embryonic development (16 mm. carapace length) the pila supraglandularis fuses along its whole length with the crista longitudinalis septi and forms a sloping roof extending ventro-laterally from the septum dorsal to the glandula nasalis media.

The glandula nasalis media lies with its posterior end in the anterior half of the foramen praepalatinum and extends obliquely forward and dorsally, parallel to the septum, beneath the pila supraglandularis. The gland opens at its anterior end into the olfactory sac ventral to the anterior end of the crista longitudinalis septi.

The cartilago paraseptalis is a short plate forming the ventro-median boundary of the fenestra basalis. It is continuous in front with the floor of the capsule which exhibits ventrally in its posterior part a moderately deep groove between its two halves. The cartilago paraseptalis accordingly exhibits a ventro-median and a dorso-lateral face at its anterior end, but toward its posterior free end it is rotated slightly so that the faces are directed

medially and laterally and the cartilages of the two sides are nearly parallel. The dorsal margin of the cartilago paraseptalis lies slightly above the level of the ventral margin of the septum; further anteriorly, where it is more nearly horizontal, its medio-dorsal margin is at the level of the lower margin of the septum. The postero-dorsal angle of the paraseptal cartilage comes to lie in contact with the planum antorbitale for a very short space so that the fenestra basalis is almost completely surrounded by cartilage. It fails, however, to fuse with the planum antorbitale, as in *Lacerta* until a much later stage (*c.p.*, fig. 10).

The cartilago ectochoanalis is a posterior prolongation of the solum nasi which forms a short process ventral and lateral to the choana, with its dorsal surface concave and ventral surface convex, forming thus a trough-like projection. It is differentiated from the cartilago paraseptalis only by a shallow incision at its posterior end.

The fenestra basalis through which the choana passes posteriorly is vertical and transverse in position and is situated below the level of the ventral margin of the septum interorbitale. In the stage modelled the fenestra is not completely separated by cartilage from the fissura orbitonasalis because of the failure of the cartilago paraseptalis and planum antorbitale to fuse dorsally. The ventro-lateral margin of the fenestra projects posteriorly as the short cartilago ectochoanalis, above which the lateral margin of the fenestra is incised to form the broad sinus nasalis posterior. The medio-ventral margin is likewise incised to form the sinus paraseptalis. The fenestra is oval with the long axis oblique from dorso-medial to ventro-lateral, and with its dorsal end more acute than the ventral. In the *Emys* embryo modelled the dorsal end of the fenestra is not closed by cartilage but by a mass of dense connective tissue between the planum antorbitale and cartilago paraseptalis.

The two fenestrae narinae together form a large round foramen which, as it were, truncates the nasal capsule anteriorly. Unlike that of *Lacerta*, which faces ventrally and laterally, that of *Emys* is directed anteriorly in a vertical transverse plane. Dorsally

and ventrally from each foramen the capsular wall is produced in an anterior direction into a short process, the dorsal one of which is slightly stronger than the ventral. Laterally the margin of the foramen is incised to accommodate the duct of the *glandula nasalis externa* which passes medially to open into the nasal sac. The *fenestra narina* lies in the ventral half of the capsule so that the entire front wall of the *pars respiratoria* is lacking. The *septum nasi* ends freely in front on a level with the lateral margins of the *fenestra* so that the two *fenestrae* are completely separated from each other.

A small *fenestra epiphaniale* in the antero-dorsal portion of the *paries nasi*, near the point at which the latter passes over into the *tectum nasi*, is the only perforation of the *paries nasi* in the stage modelled. Through this *fenestra* the *nervus lateralis nasi* of the ophthalmic branch of the trigeminus passes to the exterior of the capsule.

The *foramen praepalatium* is an elongated slit separating the *solum nasi* from the *septum nasi* for a space posterior to the *lamina transversalis anterior*. It is divided by a projection of its lateral margin into a smaller anterior and larger posterior lobe. The indentation of its lateral margin is produced by the posterior end of the *pila supraglandularis* which is attached at this point to the *solum nasi* as already described.

The *fenestra olfactoria* (*f.ol.*, fig. 24) as already mentioned, slopes ventrally in front from the antero-ventral margin of the *planum suprasedale* to the *tectum nasi*. It is a long oval, bounded medially by the free margin of the *septum nasi* and laterally by the *commissura spheno-ethmoidalis*. The planes of the two *fenestrae* are slightly inclined toward each other because the *septum* does not extend dorsally as far as the level of the *commissurae spheno-ethmoidales*.

The *fissura orbitonasalis* is continuous ventrally and anteriorly with the *sinus paraseptalis*. Dorsally it is separated from the *fenestra olfactoria* by only the slender *commissura spheno-ethmoidalis*. The *nervus ethmoidalis rami ophthalmici trigemini* gains access to the nasal capsule from the orbit through it.

PALATOQUADRATUM AND MANDIBLE

The palatoquadratum of *Emys* is of special interest since it has a distinct pars palatina, or processus pterygoideus; a condition which recalls the primitive one of the skull of *Sphenodon* and the *Anamnia* in general. It is entirely disconnected from the rest of the skull. The pars quadrata lies close alongside of the lateral wall of the otic capsule and the pars palatina alongside of the crista basipterygoidea, both parts, however, are separated from the median portion of the skull by at least a thin layer of non-cartilaginous tissue. The form of the pars quadrata has been very aptly compared to that of the external ear of man with the convex margin directed anteriorly and the concave margin posteriorly; accordingly the anterior portion of the pars quadrata is much higher dorso-ventrally than the posterior portion. It is of about the same size as the otic capsule so that it conceals the latter somewhat when viewed from the side. Its ventral portion, the pars articularis, lies well below the level of the basal plate so that its dorsal margin lies also somewhat below that of the otic capsule. In its posterior portion, the pars quadrata, fits into the broad, shallow groove formed on the lateral wall of the otic capsule by the crista parotica and the portion of the prominentia canalis semicircularis lateralis which lies dorsal to it. The median wall of the pars quadrata has a nearly vertical position so that it is much further removed from the capsule ventrally than dorsally where the lateral semicircular canal projects. In the comparatively narrow space between the quadratum and the otic capsule are situated the arteria carotis interna and vena capitis lateralis which extend, parallel to each other, obliquely from postero-ventral to antero-dorsal, as has already been described by Noack.

The pars quadrata may be differentiated into two distinct portions, a postero-dorsal one which encloses an extension of the tympanic cavity, regarded by Hasse as the homologue of the antrum mastoideum of human anatomy (pars mastoidea), and an antero-ventral portion which lacks the outer wall of the former and so exhibits an imperfect cup shape (pars articularis). The pars mastoidea of the quadratum forms a hollow cone, flattened from side to side, with its apex directed posteriorly and its base

directed anteriorly. The lateral wall of this region exhibits an irregular fenestra which indicates simply the beginning of the absorption of the cartilage under the influence of the adjacent squamosum. The base of this portion of the quadratum opens freely into the postero-dorsal portion of the pars articularis dorsal to the incisura columellae. The pars articularis is continuous with the pars mastoidea by means of the median wall and the dorsal margin of the latter which is rolled over laterally and ventrally to a slight extent to enclose partially the concavity of this region from above. The anterior and ventral margins of the anterior half of the quadratum are rolled over laterally to form a rim enclosing the tympanic cavity in front and below. Ventrally this rim becomes somewhat heavier and higher to form the processus articularis. The anterior half of the pars quadrata is much higher than the posterior, so that the processus articularis projects well below the ventral margin of the posterior part. Between the latter and the ventrally projecting processus articularis in front is a broad triangular sinus (incisura columellae) for the stalk of the columella.

The processus articularis bears a saddle-shaped articular surface for the articulation of the lower jaw. The axis of the surface is slightly oblique, sloping ventrally and posteriorly from the medial to the lateral side.

The pars palatina arises from the anterior end of the median aspect of the pars quadrata near its ventral angle and extends anteriorly and ventrally as a gradually tapering rod, its distal end bending latero-ventrally at a right angle. Of especial interest in relation to the pars palatina is the processus ascendens. This is a slender, laterally compressed process which arises from the processus pterygoideus, about midway between its origin from the pars quadrata and its sharp lateral bend. The processus ascendens extends lateral to the foramen prooticum, but somewhat removed from it. Its free distal end is close to the ventral end of the processus descendens of the parietale. Judged by its relations to the nervus trigeminus and to the palatoquadratum, it is the homologue of the 'columella' of the kionocraniat lizards, as has been pointed out by Filatoff ('06). It extends

medial to the rami maxillaris and mandibularis of the nervus trigeminus and lateral to the ramus ophthalmicus. As development proceeds this process is reduced in size, becoming replaced by the very variable epipterygoideum of the adult.

The quadratum stands in very close relationship topographically with the columella auris as has already been shown, the posterior margin being deeply incised for the accommodation of the slender stalk of the columella as it passes horizontally from the otic capsule as far laterally as the outer wall of the quadratum where it is expanded to form a mushroom like plate which partially fills the cup-like ventral half of the quadratum.

COLUMELLA AURIS

The columella auris in the embryo modelled consists of a single cartilaginous rod in which only a trace of its origin from two centers is preserved in the fact that the chondrification of the external end is very distinctly less advanced than of the rest. In earlier stages, however, the stapes and extracolumella, which together form the columella auris, are very evidently distinct. Topographically are to be distinguished the foot plate, stalk, and insertion piece, of which the first two belong to the stapes and the last to the extracolumella.

The foot plate is triangular and fits closely into the fenestra vestibuli, resting with its ventral margin upon the planum basale. The stalk arises from near the anterior angle of the foot plate, gradually assuming its slender, cylindrical form and extending at right angles to it in a lateral direction. It is slightly sigmoid, with its lateral extremity further forward than its median extremity. It extends laterally across the cavum tympanicum ventral to the vena capitis lateralis and nervus facialis and resting in the incisura columellae of the quadratum as already described. It passes into the median surface of the insertion piece somewhat dorsal to the center of the latter. The insertion piece is mushroom-shaped with its medial surface flat and lateral aspect convex. Its plane is vertical and continuous with the lateral wall of the quadratum. It aids in closing the cavum tympanicum laterally.

Of especial interest in connection with the extracolumella is the processus interhyalis described by Bender ('11) in *Testudo* and the present writer (Kunkel '12) in *Emys*, extending from the postero-ventral corner medially and ventrally. From the apex of the processus interhyalis a strand of dense connective tissue extends ventro-medially towards the lateral aspect of the pars retroarticularis of Meckel's cartilage as Fuchs ('07) has described. This process, in earlier stages, is distinct from the extracolumella as the interhyale.

The origin of the columella auris from two separate centers is also clearly shown in an embryo of 7 mm. carapace length. As I have shown in an earlier paper (Kunkel '12) in this embryo the insertion piece, corresponding to the extracolumella, is distinct from the stalk, which, together with the foot plate, represents the stapes (*col.*, fig. 21). Noack's conclusion ('07) that the columella of chelonians is a derivative of the capsular wall is not confirmed by my observations. In one of my earlier stages (carapace length, 5.2 mm.) the blastema of the columella is distinctly in the prechondrial stage while that of the capsule has not proceeded so far. In this embryo the stapes extends medially as far as the lateral wall of the otic sac and is represented by a mass of prechondrium far in advance in development of any in its immediate neighborhood.

The relation of the nervus facialis to the columella, I find is essentially as Noack has described. The ramus hyomandibularis extends caudally in a straight line from the ganglion geniculi, dorsal to the stem of the columella, to the muscles which it innervates. Almost immediately behind the columella it gives off laterally the chorda tympani which passes first in a lateral direction as far as the quadratum and then curves forward, crossing the columella on its dorsal side, and then turns ventrally in front of the Eustachian tube eventually to reach the mandible.

Meckel's cartilage is of strong form, tapering regularly from the condyle anteriorly. In cross section in front of the condyle it is elliptical with the long axis of the ellipse oblique from dorso-lateral to ventro-medial. At the condyle the rami of the mandible are flattened considerably as if by pressure from above so

that each one flares out laterally to form a distinct flange. The upper surface of the condylus mandibularis is saddle-shaped to fit into the processus articularis of the palatoquadratum. The processus retroarticularis is very poorly developed, extending only a very short distance behind the condyle as a somewhat laterally compressed keel. The distal ends of the two rami of the mandible are united by a strong symphysis in the form of a moderately thin horizontal plate of triangular form which extends behind the anterior ends of the rami and fills up the angle made by the two. An independent 'basimandibular' element, such as Parker ('80) describes in *Chelone*, is not present. In earlier stages than that modelled, the symphysis is much shorter, although sections in this region show the rami to be widening medially just behind their union to fuse finally and increase the strength of the symphysis.

In the stage modelled, the proximal end of Meckel's cartilage has not yet begun to ossify to form an articulare. This ossification becomes evident for the first time in an embryo having a carapace length of 13.5 mm.

In the earliest stage which I have studied (carapace length, 4.7 mm.) Meckel's cartilage is present as a cylindrical rod of prechondrium which tapers gradually as it proceeds from its articulation to its distal (anterior) end. At this stage the rami do not meet in a symphysis. The processus retroarticularis is relatively longer than at a later stage, possibly because the condylus, in becoming larger, usurps a portion of the original pars retroarticularis.

HYOID AND VISCERAL ARCHES

The development of the hyoid and visceral arches of *Emys* has already been described by Fuchs ('10) to whose account I can add from my own study of this region only a few points.

The corpus hyale in the stage modelled is simple and of the pentagonal form usual in the adult chelonians. The apex is directed anteriorly and from its lateral margins project three pairs of processes. It is somewhat concave dorsally and convex ventrally to form a shallow cup with a thickened rim in which the

larynx rests. The anterior apex of the corpus is produced anteriorly to form a conical processus lingualis which exhibits on its dorsal surface at its proximal end a shallow longitudinal groove representing the original space between the pair of processes of the corpus from which the processus lingualis has developed. The two antero-lateral angles of the corpus (processus lateralis anterior) are continuous laterally and posteriorly with the very short cornua hyalia. The processus lateralis intermedius is small and separated from the cornu branchiale primum. The processus lateralis posterior is continuous at this stage with the stout cornu branchiale secundum. The cornua hyalia may be differentiated from the processus lateralis anterior by a deep cleft which extends anteriorly from behind so that the cornu itself is represented by a broad, flat, stump which projects posteriorly and is thicker at its distal than at its proximal end.

The cornua branchialia prima are the longest of all the cornua and are long cylindrical rods, tapering very gradually toward their distal ends, and curved at first slightly in a postero-lateral direction and then toward their distal ends in simply a dorsal one. At their extreme distal ends they bend sharply in a medial direction and anteriorly to form short hooks whose free ends are directed forwards. These rods are in close relationship to, but are not continuous with, the processes of the corpus by their proximal ends. The cornua branchialia secunda are at this stage continuous with the processus laterales posteriores. They are heavy rods of elliptical cross section, rather thicker than the cornua branchialia prima. They are parallel in general with the last mentioned and terminate with their distal ends slightly upturned some distance ventral and medial to the extremity of the cornu branchiale primum. The second pair of cornua are considerably shorter than the first and so do not extend so far posteriorly.

No trace of an entoglossum occurs in the embryo modelled, but in an older individual (carapace length, 28 mm.) I find a thin plate of cartilage just ventral to the anterior end of the corpus hyale. It extends beyond the processus lingualis and is separated from it by only a thin layer of connective tissue. It is

somewhat triangular, with the base posterior and apex in front. This cartilago entoglossalis is imbedded in a much larger mass of fibrous tissue in which some scattered cartilage cells are present. In the adult, this surrounding mass of fibrous tissue forms a conspicuous heart-shaped plate beneath the anterior end of the corpus hyale.

In the adult of *Emys*, as already known, the three pairs of cornua are distinct from the corpus hyale and are connected with it by connective tissue. Already in a young individual with a carapace length of 28 mm. the three pairs of cornua are distinct from the corpus although the cornu branchiale primum is the only part of the hyobranchial arches which exhibits an ossification. The cornu hyale becomes segmented from the corpus hyale slightly earlier than does the cornu branchiale secundum; for example, in an embryo with carapace length of 13.5 mm. the separation of the cornu hyale is complete while the cornu branchiale secundum is only partially separated.

A separate epibranchiale primum (Siebenrock '99) is present as a separate triangular cartilage lying dorsal to the extreme distal end of the cornu branchiale primum in an embryo having a carapace 13.5 mm. long. It remains as a distinct cartilaginous element in the adult.

In the fully grown *Emys* the cornua and corpus hyale are ossified and only the processus lingualis, a small oval foramen in the anterior part of the corpus, the extreme distal ends of the cornua branchialia primum and secundum, and the epibranchiale primum remain chondrified.

The earliest portion of the hyobranchial apparatus to be laid down in cartilage is the cornu branchiale primum which is already chondrified in an embryo having a carapace length of 7 mm. It likewise is the earliest to show signs of ossification. In the embryo modelled, ossification has already begun in its middle portion, a short distance behind the posterior margin of the corpus hyale. The cornu hyale chondrifies later than the corpus hyale and at essentially the same time as the cornu branchiale secundum; so that in the stage modelled, although the body is completely chondrified, the cornu hyale is represented by a sepa-

rate cartilaginous center at some distance from the corpus and is surrounded by a mass of chondroblasts which is continuous with it.

The processus lingualis arises from a pair of short processes extending forward from the front margin of the corpus hyale at a short distance from each other and converging slightly toward their distal ends. In the stage modelled it is present as a single median rod of cartilage with only a slight indication on its dorsal surface, in the form of a shallow longitudinal groove, of its paired origin. Cross sections, however, prove its double nature even at a much later stage.

MEMBRANE BONES

The membrane bones are all laid down in the stage modelled with the exception of the parasphenoideum, quadrato-jugale, and the complementare of the lower jaw.

The squamosum (s., figs. 24, 25 and 26) is a thin plate of bone of irregular triangular form, exhibiting a convex lateral and a concave median face. It lies parallel to the outer side of the quadratum, overlying the posterior extension of the same, and separated from it by only a narrow layer of connective tissue. Its posterior margin projects slightly beyond the quadratum while its dorsal margin shows a tendency to curve medially to embrace the quadratum more closely. The ventral angle of the bone is also bent medially below the posterior end of the quadratum.

As the embryo increases in size the squamosum comes to lie with its postero-ventral angle resting against the crista parotica while its dorsal margin extends medially above the quadratum to come in contact with the lateral wall of the otic capsule dorsal to the prominentiae canalis semicircularis lateralis and ampullaris posterior. The anterior angle of the squamosum also comes to extend relatively further forward in older embryos.

The first appearance of a squamosum is in an embryo having a carapace length of 7 mm., at which stage it has the form of a shallow saucer in contact by its margins with the posterior extension of the quadratum.

In its relation to the quadratum, the squamosum agrees closely with that established by Thyng ('06) as the criterion of that bone.

The quadrato-jugale is not present in the stage modelled, but appears first in my next older embryo (carapace length, 13.5 mm.). In this it forms a triangular plate with its ventral angle greatly prolonged in front of the pars quadratum of the palatoquadratum. Its postero-dorsal angle lies a short distance ventral to the anterior angle of the squamosum and its postero-ventral margin follows closely the free anterior margin of the pars articularis of the palatoquadratum. Its anterior portion comes to lie ventral to the posterior end of the postfrontale and posterior to the posterior end of the zygomaticum. In older embryos the posterior end of the quadrato-jugale is overlapped externally by the squamosum.

It is of special interest in this connection that in such chelonians as *Cistudo ornata*, *Chelodina longicollis*, and *Geoemyda spinosa*, the embryonic character of the absence of a quadrato-jugale is retained through life.

The zygomaticum (*z.*, fig. 26) in the stage modelled is in the form of a long, slender plate of bone bent so that it exhibits one limb extending horizontally forward and the other obliquely postero-dorsally. At the same time the plane of the bone is somewhat twisted on its own axis so that whereas the postero-dorsal portion is sagittal, exhibiting a lateral and medial surface, the anterior portion is more nearly horizontal, exhibiting a dorsal and a ventral surface. The bone lies freely in the postorbital region quite far in front of the front margin of the pars quadrata. Together with the postfrontale, it completes the posterior margin of the orbit. Its anterior end extends forward dorsal to the hinder end of the maxillare.

The maxillare (*m.*) consists of a thin elongated plate of bone having in general a horizontal position and forming the ventrolateral margin of the anterior end of the skull. Its dorsal surface is slightly concave so that it exhibits a shallow longitudinal groove; ventrally it is strengthened by a vertical rib which grows higher toward the anterior end. The plane of the horizontal

portion of the maxillare becomes somewhat twisted at the anterior end where the bone embraces the olfactory capsule so that the dorsal surface becomes strongly inclined toward the median plane of the head. There may be distinguished three definite regions, the processus palatinus, processus alveolaris, and processus praefrontalis. The processus palatinus is horizontal, exhibiting a dorsal and a ventral surface; it is of nearly uniform thickness throughout, but becomes slightly thicker at the anterior than at the posterior end. Near its posterior end it is perforated by a small foramen for the ramus maxillaris nervi trigemini, which passes from above through the foramen to continue forward on the ventral side of the maxillare, lateral to the processus alveolaris. This latter has a vertical position, springing from the mid-ventral line of the processus palatinus and increasing in height from posterior to anterior. For the anterior one-third of its length the maxillare is made up entirely of the processus praefrontalis which is continuous ventrally with the anterior end of the processus alveolaris and dorsally with the processus palatinus. At the junction of the processus palatinus and praefrontalis are several irregular foramina (foramina alveolaria externa superiora.)

The processus praefrontalis has a concave median and convex lateral surface and embraces the posterior two-thirds of the olfactory capsule ventro-laterally. Its antero-median margin is hollowed slightly in order to accommodate the praemaxillare. The dorsal margin of the processus praefrontalis lies in the same plane with and rather close to the ventral margin of the prae-frontalis.

The processus palatinus is overlapped dorsally for a very short distance by the anterior end of the zygomaticum so that the two bones together complete the margin of the orbit posteriorly and ventrally. It lies in the same horizontal plane with the palatinum which comes to lie in close relation to it by its anterior end.

The praemaxillare (*prm.*) is of small irregular form situated ventral to the olfactory capsule in the region of the foramen prae-palatinum and medial to the anterior end of the maxillare. Like

the maxillare it exhibits a processus palatinus and a processus alveolaris. The former is horizontal, exhibiting a dorsal and a ventral surface, and is prolonged anteriorly at its median margin to form a short process which extends nearly as far as the anterior margin of the solum nasi and which may represent a very rudimentary processus praenasalis. Even in the adult *Emys*, however, the praemaxillare remains wholly ventral to the olfactory capsule and does not extend anterior to it to separate the two fenestrae narinae from each other as in *Lacerta*, for example. The processus alveolaris is a vertical plate extending from the ventral surface of the processus palatinus in an oblique direction from postero-lateral to antero-medial.

The postfrontale (*pst.*) has the form of a triangular plate with an apex directed forward and its upper margin horizontal; its antero-ventral margin is slightly concave and thickened by a laterally projecting flange which arises near the ventral angle of the bone and extends to the anterior apex. It lies in the space between the quadratum posteriorly and the orbit anteriorly, lateral to the parietale. It approaches the posterior extremity of the zygomaticum with its ventrally projecting angle, and the anterior margin of the quadratum in its most lateral portion with its posterior margin. It has no share whatever in the formation of the cranial wall.

The parietale (*par.*) and frontale (*f.*) have essentially the same relations to the chondrocranium that they do in *Lacerta*; that is, their position is wholly lateral and in the stage of *Emys* modelled they do not begin to roof in the cranial cavity. The parietale is large and triangular in form exhibiting long dorsal and antero-ventral margins, a short posterior one, and a lateral and a medial face. Its postero-dorsal angle is prolonged slightly to form a small processus occipitalis, extending dorsal to the otic capsule for a short distance. Ventrally the parietale is prolonged to form a processus inferior which extends downward lateral to the pila prootica to come into close relationship with the free end of the processus ascendens of the palatoquadratum. The processus inferior thus comes to enclose a space external to the primordial cranial cavity so that the latter is increased in size on the sides. This space is the cavum epiptericum.

The antero-ventral margin of the parietale lies parallel to the free margin of the planum supraseptale at a short distance dorsal and lateral to it, while its anterior angle comes to lie medial to the posterior end of the frontale. The posterior margin of the parietale rests with its projecting processus occipitalis against the anterior cupola of the otic capsule.

The frontale is a narrow, curved plate that continues the contour of the parietale forward. Its ventral margin, like that of the parietale, lies parallel to the free margin of the planum supraseptale and completes the orbit dorsally. Its posterior end overlaps laterally the anterior end of the parietale and its extreme anterior end is embraced by the dorsal extremity of the praefrontale.

In contrast to the condition in *Lacerta* the frontale in *Emys* extends less far posteriorly so that it fails to be overlapped by the anterior extremity of the postfrontale. The frontale at this stage of development is relatively small, its place being taken to a certain extent by the extremely large praefrontale in front and the unusually large parietale behind.

The praefrontale (*pf.*) or lacrimale is very large, completing the orbit anteriorly and embracing the greater part of the olfactory capsule. In form it exhibits a vertical plate which extends beyond and lateral to the antero-ventral end of the frontale, and a larger oblique portion lateral to the olfactory capsule and lying dorsal to the anterior portion (processus praefrontalis) of the maxillare. The anterior margin of the vertical limb of the praefrontale becomes very thick, so that there is a distinct anterior, as well as a lateral and a medial face. It may be that this thickening of the dorsal portion of the praefrontale stands in connection with the fact that a nasale is lacking.

The pterygoideum (*pt.*) is strongly developed in the stage of *Emys* modelled, and exhibits clearly many of the characteristics of the adult form. It is a long, slender bone with ventral face extending horizontally and a longitudinal crest, the crista pterygoidea (*c.pt.*, fig. 20), extending dorsally, which diminishes in height from posterior to anterior and separates the fossa supra-ptyerygoidea, lying lateral to it, from the sulcus cavernosus on

its median side. At its anterior end the crista pterygoidea disappears and the pterygoideum extends forward as a small horizontal process between the posterior end of the platinum laterally and the fused trabeculae medially. Near the anterior end of the lateral margin of the pterygoideum a conspicuous triangular process—the processus ectopterygoideus—projects laterally beneath the distal end of the processus palatinus of the palatoquadratum. Its posterior end lies with the crista pterygoidea between the base of the processus pterygoideus of the palatoquadratum laterally and the projecting antero-lateral margin of the planum basale, coming to lie in close relation to the rudimentary cartilago articularis (*c.a.*, fig. 8). The body of the pterygoideum lies in a plane ventral to the planum basale, extending laterally beneath the processus pterygoideus of the palatoquadratum and medially beneath the processus basiptyerygoideus to enclose from the ventral side a space in which run the ramus palatinus n. facialis and ramus communicans n. facialis cum glossopharyngeo, as well as the arteria carotica interna, which lie in the sulcus cavernosus on the median side of the crista pterygoidea.

The palatinum (*pal.*) is a flat, triangular plate with its apex directed posteriorly and base anteriorly. It lies at the same level as the processus palatinus of the maxillare and occupies the space in the roof of the mouth between the maxillare, vomer, and pterygoideum. Its posterior end lies external to the anterior prolongation of the pterygoideum and in front of the processus ectopterygoideus. The plane of the palatinum is inclined so that its median margin is somewhat dorsal to its lateral margin.

The parasphenoideum is wanting in the stage modelled, but in an older embryo (carapace length, 13.5 mm.) it is present as a small tripartite plate lying ventral to the region of the fenestra hypophyseos. Its anterior median process extends as far forward as the front margin of the fenestra while its postero-lateral processes extend scarcely as far laterally as the lateral margins of the fenestra.

The parasphenoideum lies immediately in front of and partially embraces on the two sides the stalk of the hypophysis at this stage. It is also of interest that a small lamella of bone extends horizontally forward from the front margin of the crista sellaris to occlude partially the fenestra hypophyseos. In an older embryo having a carapace length of 16 mm. this lamella from the crista sellaris has extended itself further forward and fused completely with the parasphenoideum so that only a very small opening is left between the posterior processes of the parasphenoideum and this lamella for the accommodation of the hypophysial stalk. In the meanwhile the ossification of the crista sellaris, which becomes the basisphenoideum, has set in.

The basisphenoideum of the adult Emtys must therefore be composed of two parts, an anterior part not preformed in cartilage and a posterior part laid down originally in the crista sellaris as a replacing bone.

The vomer (*v.*) in the stage modelled is an unpaired bone having the form of a shallow trough which lies with its dorsal concave face ventral to the anterior end of the septum interorbitale and separated from it by a thin layer of connective tissue. In form the vomer tapers slightly in a horizontal plane toward the anterior end and also grows thicker dorso-ventrally.

In an embryo having a carapace length of 8.5 mm. the vomer shows clearly its paired nature, being represented by a pair of thin lamellae set at an angle to each other so that they fit about the ventral edge of the septum interorbitale between the posterior opening of the ductus naso-pharyngeus behind and the cartilago paraseptalis in front. In an embryo having a carapace length of 13.5 mm. the vomer extends relatively further posteriorly than in the stage modelled and its lateral margins lie close to the median margins of the two palatina which, however, come to lie rather dorsal to the vomer.

The investing bones of the adult lower jaw are all present in the stage modelled except the complementare which appears first in an embryo having a carapace length of 13.5 mm. They may be designated as the dentale, angulare, supra-angulare, and goniale. An operculare is lacking as in the adult. The arti-

culare, an ossification of the proximal end of Meckel's cartilage, is not apparent in an *Emys* embryo having a carapace length of 11 mm.

The dentale (*d.*) has the form of a long, slender plate exhibiting in general a concave medial and convex lateral surface. It is provided with flattened flanges extending both laterally and medially from the dorsal margin. It lies lateral to Meckel's cartilage for almost the entire distance from the anterior, distal end to the fovea articularis, overlapping with its posterior end the anterior end of the supra-angularare so that the latter is interposed between the dentale and Meckel's cartilage.

By reason of the medial projection of the dorsal margin of the dentale, this bone comes to arch in dorsally the sulcus primordialis which accommodates Meckel's cartilage. Beneath the lateral flange of the dentale extends the portio alveolaris inferior of the ramus mandibularis n. trigemini which gains access to this groove (sulcus alveolaris inferior) from the sulcus primordialis through the foramen canalis alveolaris inferioris. This foramen is situated near the posterior end of the dentale immediately ventral to the thickened dorsal margin. In older embryos the sulcus alveolaris inferior becomes converted into the closed canalis alveolaris inferior by the further rolling over of the lateral margin of the dorsal flange.

The supra-angularare (*sa.*) is a small elongate plate of bone which lies along the lateral aspect of Meckel's cartilage in its proximal portion. It exhibits a slender posterior projection which lies along the lateral side of the fovea articularis. The anterior third of the supra-angularare at this stage is overlapped laterally by the posterior end of the dentale. The ramus recurrens cutaneus mandibulae from the mandibular branch of the trigeminus passes obliquely backward along the dorsal margin of the supra-angularare and somewhat medial to it.

The angularare (*a.*) is a long slender plate of bone lying on the ventral side of the posterior end of Meckel's cartilage in the region of the supra-angularare. It forms, accordingly, the floor of the sulcus primordialis in its posterior part. It is much shorter than the supra-angularare and extends neither as far anteriorly

nor posteriorly as the latter. Its antero-lateral margin lies in close proximity to the ventral margin of the dentale.

The goniale (*g.*) is a thin, elongated plate of bone situated along the median aspect of Meckel's cartilage, from the posterior end of the latter for one-third of its length. It exhibits a convex median and a concave lateral aspect to fit more closely about the posterior end of Meckel's cartilage medially. Its postero-dorsal angle lies along the median aspect of the fovea articularis.

Of especial interest in connection with the goniale is the relation of the chorda tympani. In the embryo modelled the nerve enters the goniale at its posterior extremity by means of the canalis gonialis which extends forward for a short distance and opens anteriorly on the lateral aspect of the goniale as a well marked sulcus so that the chorda tympani reaches the canalis primordialis and extends further forward in this canal between the goniale on the median side and Meckel's cartilage on the lateral side. In an older embryo (carapace length, 13.5 mm.) a canalis gonialis is wanting. Instead of this the chorda tympani is lodged in a groove on the median side of the goniale. The nerve passes obliquely forward and ventrally and for a short space lies along the ventral margin of the goniale. The latter then becomes suddenly wider by a ventrally projecting extension which is lateral to the nerve. For a considerable distance the chorda tympani is thus separated from Meckel's cartilage by the goniale. A foramen finally allows the nerve to pass into the sulcus primordialis.

A third relationship of the chorda tympani and goniale was encountered in an embryo having a carapace length of 16 mm. In this individual the nerve entered the canalis gonialis from the lateral aspect of the bone and both before and after entering the canalis gonialis it lay in a deep groove communicating with the sulcus primordialis.

The complementare is not yet laid down in the embryo modelled. In an embryo having a carapace 13.5 mm. long, however, it is present as a triangular plate of bone lying dorsal to the goniale with its anterior end somewhat further forward than that of the goniale. It lies medially to the ramus mandibularis n. trigemini.

SUMMARY

The principal points which I would emphasize in conclusion are as follows:

1. The chondrocranium of *Emys* resembles closely that of *Dermochelys* and *Chelone* and, in general features, is similar to that of *Lacerta*, but is of heavier construction.

2. The planum basale is parachordal in position, surrounding the chorda dorsalis on all sides.

3. The condylus occipitalis is annular in form, a central concavity being present around the chorda dorsalis.

4. The arcus occipitales do not reach the tectum posterius distally so that the foramen occipitale magnum is not separated completely from the fissura metotica.

5. There are three foramina spino-occipitalia present at the stage modelled, the two anterior of which fuse later.

6. The tectum posterius is strongly developed, exhibiting a processus ascendens of large size and a stout processus posterior which limits the foramen occipitale magnum dorsally.

7. The fissura metotica becomes wide at its antero-ventral end at which point the nervus vagus and vena jugularis leave the cranial cavity.

8. The planum basale projects laterally beyond the lateral portions of the occipital region as the crista inferior which thereby forms the ventral side of the sulcus supracristularis.

9. The fenestra cochleae opens into the sulcus supracristularis at its anterior end (recessus scalae tympani) immediately in front of the fissura metotica.

10. The fenestra cochleae is the posterior opening of the canalis perilymphaticus which has a horizontal direction and opens into the cavum cochleae on the median wall of the latter.

11. A canalis hypoperilymphaticus is found in embryos older than that modelled, passing parallel and ventral to the canalis perilymphaticus and communicating between the recessus scalae tympani posteriorly and the cavum cochleae anteriorly. It contains nothing but loose connective tissue. It develops anteriorly from its posterior end.

12. The nervus glossopharyngeus passes through the cavity of the otic capsule in the sulcus glossopharyngeus which is situated on the posterior wall of the cavum cochleae immediately ventral to the cavum ampullare posterius. The nerve perforates the median capsular wall by means of the foramen glossopharyngei internum and the outer wall by the foramen glossopharyngei externum.

13. The cavum cochleae is developed in a posterior direction so that the nervus glossopharyngeus passes through it.

14. The cavity of the otic capsule is not divided by a septum intervestibulare as in *Lacerta*.

15. The median capsular wall is perforated anterior to the foramen glossopharyngei internum by the foramina acustica anterius and posterius and endolymphaticum; besides these are one or two smaller foramina one of which is for a blood vessel.

16. The foramen facialis lies anterior to the otic capsule and opens laterally into a depression, the fovea geniculi.

17. The foramen abducentis passes horizontally forward through the base of the pila prootica.

18. The fenestra prootica is large and unclosed dorsally because of the absence of a taenia marginalis to unite the distal end of the pila prootica and the otic capsule.

19. The fenestra hypophyseos is large and accommodates, besides the hypophysis cerebri, the arteriae carotides internae as they pass into the cavity of the cranium.

20. The fenestra metoptica, for the accommodation of the nervi oculomotorius and trochlearis, is in the form of a narrow slit and, like the fenestra prootica, is unclosed dorsally.

21. The planum basale projects laterally in the region of the crista sellaris beyond the attachment of the otic capsule to form the crista basipterygoidea which is apparently homologous with the processus basipterygoideus and with which articulates a rudimentary imperfectly chondrified cartilago articularis. The ramus palatinus nervi facialis passes ventrally from the ganglion geniculi a short distance behind the cartilago articularis and then extends anteriorly on the median side of the cartilago articularis in the sulcus cavernosus.

22. The septum interorbitale is imperforate and is quite evidently of double origin since in younger stages than that modelled it is seen in cross section to have a distinct Y-form, the two limbs of the Y being parallel to each other and then suddenly diverging to form the planum suprasedale.

23. The fenestra optica is large and is situated in front of the foramen ophthalmicum through which the arteria ophthalmica passes into the region of the orbit of the eye. The two foramina ophthalmica are separated from each other by the subiculum infundibuli.

24. The septum interorbitale passes without interruption forward into the septum nasi which divides the ethmoidal region into two symmetrical halves.

25. The olfactory capsule is of compact form and is composed of continuous plates of cartilage not separated by extensive foramina.

26. The capsule is bent somewhat ventrally as is indicated by the plane of the fenestra olfactoria which is not horizontal, as in *Lacerta*, but inclines downward in front; and also by the fact that the fenestra basalis is situated below the level of the ventral margin of the septum interorbitale.

27. The olfactory capsule is connected with the septum posteriorly only by the slender commissurae spheno-ethmoidales which bound the fenestrae olfactoriae laterally and separate them from the fissurae orbitonasales.

28. The fissura orbitonasalis extends the entire length of the posterior cupula of the capsule, as in the *Lacertilia*, the planum antorbitale being entirely separated from the septum.

29. The nervus ethmoidalis passes into the capsule from the orbit through the fissura orbitonasalis.

30. The plane of the fenestra narina is transverse so that the fenestra faces directly anteriorly.

31. The lamina transversalis anterior is represented by the portion of the floor of the capsule in front of the foramen praepalatinum.

32. The shortness of the cartilago paraseptalis of the stage modelled is a secondary condition, since in earlier stages the fora-

men praepalatinum is not closed posteriorly by a fusion of the cartilago paraseptalis with the septum, but extends freely posteriorly for a considerable distance.

33. The septum nasi in the stage modelled is continuous with the solum nasi except in the region of the foramen praepalatinum.

34. The cartilago paraseptalis, limiting the fenestra basalis medially, does not fuse with the planum antorbitale dorsally so that in the stage modelled the fenestra basalis is not completely enclosed with cartilage.

35. A rudimentary concha is present in the form of a shallow longitudinal infolding of the paries nasi. In older stages this is increased in height by a thickening of the wall which projects in a medial direction.

36. The septum nasi is thickened by means of a longitudinal crest, the crista longitudinalis septi, which separates the pars respiratoria from the pars olfactoria of the olfactory sac.

37. A slender rod, the pila supraglandularis, attached posteriorly to the septum in the region of the foramen praepalatinum, extends forward parallel to the crista longitudinalis septi above the glandula nasalis media and ends freely in front a short distance behind the level of the fenestra narina.

38. The septum nasi is provided with a small fenestra very near its dorsal margin in the region of the fenestra olfactoria.

39. The prenasal cartilage is present as a median ridge on the ventral side of the anterior part of the capsule.

40. A small foramen epiphaniale is present.

41. The palatoquadratum is entirely separate from the cranium and exhibits a distinct processus pterygoideus which in turn supports a processus ascendens which is the homologue of the 'columella' of the Lacertilia.

42. The pars quadrata of the palatoquadratum may be differentiated into a large hollow pars mastoidea, enclosing an extension of the tympanic cavity, and the pars articularis.

43. The posterior margin of the quadratum is deeply incised by the incisura columellae for the accommodation of the columella auris.

44. The columella auris originates wholly external to the otic capsule and is made up of stapes and extracolumella. The foot plate occupies the fenestra vestibuli and rests upon the crista substapedialis; the stalk of the columella is slender and slightly sinuous; the extracolumella is mushroom-shaped.

45. A distinct processus interhyalis extends medially and ventrally from the ventral angle of the extracolumella.

46. The ramus hyomandibularis nervi facialis extends posteriorly dorsal to the columella and at the level of the latter gives off the chorda tympani in a lateral direction. This nerve then curves anteriorly, crossing the columella dorsally, and bends ventrally in front of the Eustachian tube.

47. Meckel's cartilage is strongly developed. The two cartilages meet by a strong symphysis anteriorly. The processus retroarticularis is poorly developed.

48. The corpus hyale is pentagonal, with the apex directed anteriorly. The short cornua hyalia are not segmented from the corpus. The cornua branchialia primum and secundum are both large and segmented from the corpus. A separate epibranchiale primum is present.

49. The processus lingualis arises as a pair of anteriorly directed rods from the anterior end of the corpus hyale, but at the stage modelled the two have grown together.

50. The cartilago entoglossalis appears for the first time at a later stage than that modelled.

51. The squamosum is a thin plate of bone lying parallel to the outer side of the quadratum.

52. The quadrato-jugale is not laid down in the embryo till a later stage than that at which most of the investing bones are laid down.

53. The zygomaticum, together with the postfrontale, completes the orbit of the eye posteriorly.

54. The maxillare, as in the adult, exhibits a processus palatinus, a processus alveolaris, and a processus praefrontalis. Its posterior end is perforated by a foramen for the passage from above of the ramus maxillaris nervi trigemini which continues forward lateral to the processus alveolaris.

55. The praemaxillare is of small size, situated entirely ventral to the olfactory capsule.

56. The parietale and frontale are wholly lateral in position and do not begin at this stage to arch in the cranial cavity dorsally. The parietale exhibits a large processus inferior which extends downward, lateral to the pila prootica, to come at length into close relation with the free end of the processus ascendens palatoquadrati. A cavum epiptericum is hereby formed.

57. The praefrontale is exceedingly large and becomes very thick at its anterior margin, dorsal to the olfactory capsule.

58. The pterygoideum exhibits a pronounced crista pterygoidea on its dorsal side which separates the fossa suprapterygoidea from the sulcus cavernosus.

59. The palatinum and vomer complete the roof of the mouth at the stage modelled although later a separate parasphenoideum is present in the region of the fenestra hypophyseos. The vomer is unpaired but is derived from a paired condition.

60. In the lower jaw all the investing bones of the adult except the complementare are present at the stage modelled.

61. Along the lateral side of the dentale is the sulcus alveolaris inferior which becomes converted into the canalis alveolaris inferior of older embryos.

62. The goniale in the stage modelled is penetrated by the canalis gonialis in which the chorda tympani passes from the posterior end of the goniale obliquely forward and laterally to attain the canalis primordialialis.

BIBLIOGRAPHY

No attempt is made in this bibliography to give a complete list of the works on the morphology of the skull, as such a list is to be found in Gaupp, 1905 b, and more specifically for the chelonian skull in Nick, 1912. Only the more recent and important works which are referred to in the body of the paper are here mentioned.

- BENDER, O. 1911 Ueber Herkunft und Entwicklung der Columella auris bei *Testudo graeca*. *Anat. Anz.*, Bd. 40, pp. 161-177.
- BOJANUS, L. H. 1819 *Anatome Testudinis Europaeae*. Vilnae, 1819-1821.
- FILATOFF, D. 1906 Zur Frage über die Anlage des Knorpelschädels bei einigen Wirbeltieren. *Anat. Anz.*, Bd. 29, pp. 623-633.
- 1907 Die Metamerie des Kopfes von *Emys lutaria*. Zur Frage über korrelative Entwicklung. *Morph. Jahrb.*, Bd. 37, pp. 289-396.

- FUCHS, H. 1906 Untersuchungen über die Entwicklung der Gehörknöchelchen, des Squamosums, und des Kiefergelenkes der Säugetiere. Arch. f. Anat. u. Physiol., Anat. Abt., Supplement-band, pp. 1-90.
- 1907 a Untersuchungen über Ontogenie und Phylogenie der Gaumenbildungen bei den Wirbeltieren. Erste Mitteilung. Ueber den Gaumen der Schildkröten und seine Entwicklungsgeschichte. Zeitschr. f. Morph. u. Anthrop., Bd. 10, pp. 409-463.
- 1907 b Ueber die Entwicklung des Operculums der Urodelen und des Distelidiums ('Columella' auris) einiger Reptilien. Verhandl. d. anatom. Gesellsch., 21 Versamml., pp. 8-31.
- 1907 c Ueber das Hyobranchialskelett von *Emys lutaria* und seine Entwicklung. Anat. Anz., Bd. 31, pp. 33-39.
- 1910 Ueber das Pterygoid, Palatinum und Parasphenoid der quadrupeden, insbesondere der Reptilien und Säugetiere, nebst einigen Betrachtungen über die Beziehungen zwischen Nerven und Skeletteilen. Anat. Anz., Bd. 36, pp. 33-95.
- GAUPP, E. 1905 a Neue Deutungen auf dem Gebiete der Lehre vom Säugetierschädel. Anat. Anz., Bd. 27, pp. 273-310.
- 1905 b Die Entwicklung des Kopfskelettes. Hertwig's Handbuch der Entwicklungslehre, Bd. 3, Abt. 2, pp. 573-874.
- 1905 c Die Nicht-Homologie des Unterkiefers in der Wirbeltierreihe. Verhandl. d. anatom. Gesellsch., 19 Versamml., pp. 125-140.
- 1905 d Das Hyobranchialskelett der Wirbeltiere. Ergeb. d. Anat. u. Entwickl., Bd. 14, pp. 808-1048.
- 1906 Ueber allgemeine und specielle Fragen aus der Lehre vom Kopfskelett der Wirbeltiere. Verhandl. d. anat. Gesellsch., 20 Versamml., pp. 21-68.
- 1907 a Ueber Entwicklung und Bau der beiden ersten Wirbel und der Kopfgelenke von *Echidna aculeata*. Jena. Denkschr., Bd. 6, T. 2, pp. 483-538. (Semon, Zool. Forschungsreisen, Bd. 3, T. 2).
- 1907 b Hauptergebnisse der an dem Semonschen *Echidna*-Material vorgenommenen Untersuchung der Schädelentwicklung. Verhandl. d. anat. Gesellsch., 21 Versamml., pp. 129-141.
- 1908 Zur Entwicklungsgeschichte und vergleichenden Morphologie des Schädels von *Echidna aculeata* var. *typica*. Jena. Denkschr., Bd. 6, T. 2, pp. 541-788. (Semon, Zool. Forschungsreisen, Bd. 3, T. 2).
- 1910 a Das Lacrimale des Menschen und der Säuger und seine morphologische Bedeutung. Anat. Anz., Bd. 36, pp. 529-555.
- 1910 b Säugerpterygoid und Echidnapterygoid nebst Bemerkungen über das Säuger-palatinum und den Processus basipterygoideus. Anat. Hefte, Bd. 42, pp. 311-431.
- 1911 a Ueber den N. trochlearis der Urodelen und über die Austrittsstellen der Gehirnnerven aus dem Schädelraum im Allgemeinen. Anat. Anz. Bd. 38, pp. 401-444.
- 1911 b Beiträge zur Kenntnis des Unterkiefers der Wirbeltiere. I. Der Processus anterior (Folii) des Hammers der Säuger und das Goniale der Nichtsäuger. Anat. Anz., Bd. 39, pp. 97-135. II. Die Zusammensetzung des Unterkiefers der Quadrupeden. Anat. Anz., Bd. 39, pp. 433-473.

- HASSE, C. 1873 Das Gehörorgan der Schildkröten. Hasse's Anatomische Studien, Bd. 1, pp. 225-329.
- HOFFMANN, C. K. 1890 Schildkröten. Bronn's Klassen und Ordnungen, Bd. 6, Abt. 3, pp. 1-442.
- HOWES, G. B. AND SWINNERTON, H. H. 1903 On the development of the skeleton of the tuatara, *Sphenodon punctatus*. Trans. Zool. Soc. London, vol. 16, pp. 1-86.
- KUNKEL, B. W. 1911 Zur Entwicklungsgeschichte und vergleichenden Morphologie des Schildkrötenschädels. Anat. Anz., Bd. 39, pp. 354-364.
1912 On a double fenestral structure in Emys. Anat. Rec., vol. 6, pp. 267-280.
- MONKS, SARAH P., 1878 The columella and stapes in some North American turtles. Proc. Amer. Phil. Soc., vol. 17, pp. 335-337.
- NICK, L. 1912 Das Kopfskelet von *Dermochelys coriacea* L. Zool. Jahrb., Abt. f. Anat., Bd. 33, pp. 1-238.
- NOACK, H. 1907 Ueber die Entwicklung des Mittelohres von *Emys europaea* nebst Bemerkungen zur Neurologie dieser Schildkröte. Arch. f. mikr. Anat., Bd. 69, pp. 457-490.
- OGUSHI, K. 1911 Anatomische Studien an der japanischen dreikralligen Lippenschildkröte (*Trionyx japonicus*). Morph. Jahrb., Bd. 43, pp. 1-106.
- PARKER, W. K. 1880 On the development of the green turtle (*Chelone viridis*, Schneid.) 'Challenger Reports,' Zool., vol. 1, part 5, pp. 1-58.
- SEYDEL, O. 1896 Ueber die Nasenhöhle und das Jacobson'sche Organ der Land- und Sumpfschildkröten. Festschr. z. 70. Geburtstag von Carl Gegenbaur, Bd. 2, pp. 385-486.
- SIEBENROCK, F. 1897. Das Kopfskelet der Schildkröten. Sitzungsber. d. kaiserl. Akad. d. Wissensch. Wien; math.-naturwissensch. Kl., Bd. 106, Abt. 1, pp. 245-328.
1899 Ueber den Bau und die Entwicklung des Zungenbein-Apparates der Schildkröten. Ann. d. k. k. naturhistor. Hofmuseums, Wien, Bd. 13, Heft 4, pp. 423-437.
- THYNG, F. W. 1906 The Squamosal bone in tetrapodous vertebrata. Proc. Boston Soc. Nat. Hist., vol. 32, pp. 387-425.
- VAN BEMMELLEN, J. F. 1896 Bemerkungen über den Schädelbau von *Dermochelys coriacea*. Gegenbaur's Festschrift, Bd. 2, pp. 277-286.
- VERSLUYS, J. 1909 Ein grosses Parasphenoid bei *Dermochelys coriacea*, Linn. Zool. Jahrb., Bd. 28, Anat. Abt., pp. 283-294.
1910 Bemerkungen zum Parasphenoid von *Dermochelys*. Anat. Anz., Bd. 36, pp. 487-495.
- VOIT, M. 1909 Das Primordialcranium des Kaninchens unter Berücksichtigung der Deckknochen. Anat. Hefte, Bd. 38, pp. 425-616.

ABBREVIATIONS

The following list of abbreviations is used throughout in the explanation of both text figures and plates.

<i>a.</i> , angulare	<i>f.a.a.</i> , foramen acousticum anterius
<i>a.c.i.</i> , arteria carotis interna	<i>f.a.p.</i> , foramen acousticum posterius
<i>a.o.</i> , arcus occipitalis	<i>f.b.</i> , fenestra basalis
<i>a.p.</i> , ampulla posterior	<i>f.b.p.</i> , fenestra basiscranialis posterior
<i>c.a.</i> , cartilago articularis	<i>f.c.</i> , fenestra cochleae
<i>c.a.a.</i> , cavum ampullare anterius	<i>f.e.</i> , foramen endolymphaticum
<i>c.a.l.</i> , cavum ampullare laterale	<i>f.ep.</i> , foramen epiphaniale
<i>c.a.p.</i> , cavum ampullare posterius	<i>f.f.</i> , foramen facialis
<i>c.b.</i> , crista basipterygoidea	<i>f.g.</i> , fovea genicularis
<i>c.b.p.</i> , cornu branchiale primum	<i>f.g.e.</i> , foramen glossopharyngei exter- num
<i>c.b.s.</i> , cornu branchiale secundum	<i>f.g.i.</i> , foramen glossopharyngei inter- num
<i>c.c.</i> , cavum cochleae	<i>f.h.</i> , fenestra hypophyseos
<i>c.d.</i> , chorda dorsalis	<i>f.m.</i> , fissura metotica
<i>c.e.</i> , cartilago ectochoanalis	<i>f.mp.</i> , fenestra metoptica
<i>c.h.</i> , cartilago hypochiasmatica	<i>f.n.</i> , fenestra narina
<i>c.hy.</i> , corpus hyale	<i>f.o.</i> , foramen ophthalmicum
<i>c.i.</i> , crista inferior	<i>f.ol.</i> , fenestra olfactoria
<i>c.l.</i> , crista longitudinalis septi	<i>f.o.m.</i> , foramen occipitale magnum
<i>c.M.</i> , cartilago Meckelii	<i>f.opt.</i> , fenestra optica
<i>cn.h.</i> , cornu hyale	<i>f.orb.</i> , fissura orbitonasalis
<i>c.o.</i> , condylus occipitalis	<i>f.p.</i> , foramen praepalatinum
<i>col.</i> , columella auris	<i>f.pr.</i> , fenestra prootica
<i>c.p.</i> , cartilago paraseptalis	<i>f.s.</i> , fossa subarcuata
<i>c.per.</i> , canalis perilymphaticus	<i>f.s.n.</i> , fenestra septi nasi
<i>c.pf.</i> , commissura praefacialis	<i>f.sp.</i> , foramen spino-occipitale
<i>c.pt.</i> , crista pterygoidea	<i>f.v.</i> , fenestra vestibuli
<i>cr.p.</i> , crista parotica	<i>g.</i> , goniale
<i>c.s.</i> , crista sellaris	<i>g.g.</i> , ganglion geniculi
<i>c.s.a.</i> , canalis semicircularis anterior	<i>g.gl.</i> , ganglion glossopharyngei
<i>c.sac.</i> , cavum sacculi	<i>g.s.</i> , ganglion semilunare
<i>c.s.l.</i> , canalis semicircularis lateralis	<i>g.v.</i> , ganglion vagi
<i>c.s.p.</i> , canalis semicircularis posterior	<i>i.c.</i> , incisura columellae
<i>c.sph.</i> , commissura sphenothmoidalis	<i>l.t.a.</i> , lamina transversalis anterior
<i>c.s.s.</i> , cavum sinus superioris utriculi	<i>m.</i> , maxillare
<i>c.st.</i> , crista substapedialis	<i>o.c.</i> , capsula otica
<i>c.t.</i> , chorda tympani	<i>p.</i> , parasphenoideum
<i>c.v.</i> , cavum vestibuli	<i>p.a.</i> , processus ascendens palatoquad- rati
<i>d.</i> , dentale	<i>pal.</i> , palatinum
<i>d.e.</i> , ductus endolymphaticus	<i>p.ant.</i> , planum antorbitale
<i>d.p.</i> , ductus perilymphaticus	<i>p.a.p.</i> , prominentia ampullaris pos- terior
<i>ec.</i> , extracolumella	
<i>f.</i> , frontale	
<i>f.a.</i> , foramen abducentis	

<i>par.</i> , parietale	<i>s.c.</i> , sulcus cavernosus
<i>p.b.</i> , planum basale	<i>sep.</i> , septum nasi
<i>pf.</i> , praefrontale	<i>s.g.</i> , sulcus glossopharyngei
<i>p.i.</i> , processus inferior	<i>s.i.</i> , septum interorbitale
<i>p.l.</i> , processus lingualis	<i>s.o.</i> , sinus oculomotorius
<i>p.met.</i> , pila metoptica	<i>s.s.</i> , sulcus supracristularis
<i>p.p.</i> , pila prootica	<i>s.s.a.</i> , septum semicirculare anterius
<i>pl.s.</i> , planum supraseptale	<i>s.s.l.</i> , septum semicirculare laterale
<i>pr.a.</i> , prominentia ampullaris lateralis	<i>s.s.p.</i> , septum semicirculare posterius
<i>pr.b.</i> , crista basipterygoideus	<i>sub.i.</i> , subiculum infundibuli
<i>pr.i.</i> , processus interhyalis	<i>t.</i> , trabecula cranii
<i>prm.</i> , praemaxillare	<i>t.n.</i> , tectum nasi
<i>pr.p.</i> , processus posterior	<i>t.p.</i> , tectum posterius
<i>pr.pt.</i> , processus pterygoideus	<i>u.</i> , utriculus
<i>pr.s.</i> , processus suprararinus	<i>v.</i> , vomer
<i>p.s.a.</i> , prominentia semicircularis anterior	<i>v.c.l.</i> , vena capitis lateralis
<i>p.sg.</i> , pila supraglandularis	<i>v.j.</i> , vena jugularis
<i>pst.</i> , postfrontale	<i>z.</i> , zygomaticum
<i>pt.</i> , pterygoideum	<i>I</i> , nervus olfactorius
<i>q.</i> , quadratum	<i>II</i> , n. opticus
<i>r.c.</i> , ramus communicans n. facialis cum glossopharyngeo	<i>III</i> , n. oculomotorius
<i>r.h.</i> , ramus hyomandibularis n. facialis	<i>IV</i> , n. trochlearis
<i>r.o.</i> , ramus ophthalmicus n. trigemini	<i>V</i> , n. trigeminus
<i>r.p.</i> , ramus palatinus n. facialis	<i>VI</i> , n. abducens
<i>s.</i> , squamosum	<i>VII</i> , n. facialis
<i>sa.</i> , supraangulare	<i>VIII</i> , n. acusticus
<i>sac.</i> , sacculus	<i>IX</i> , n. glossopharyngeus
	<i>X</i> , n. vagus
	<i>XII</i> , n. spino-occipitalis

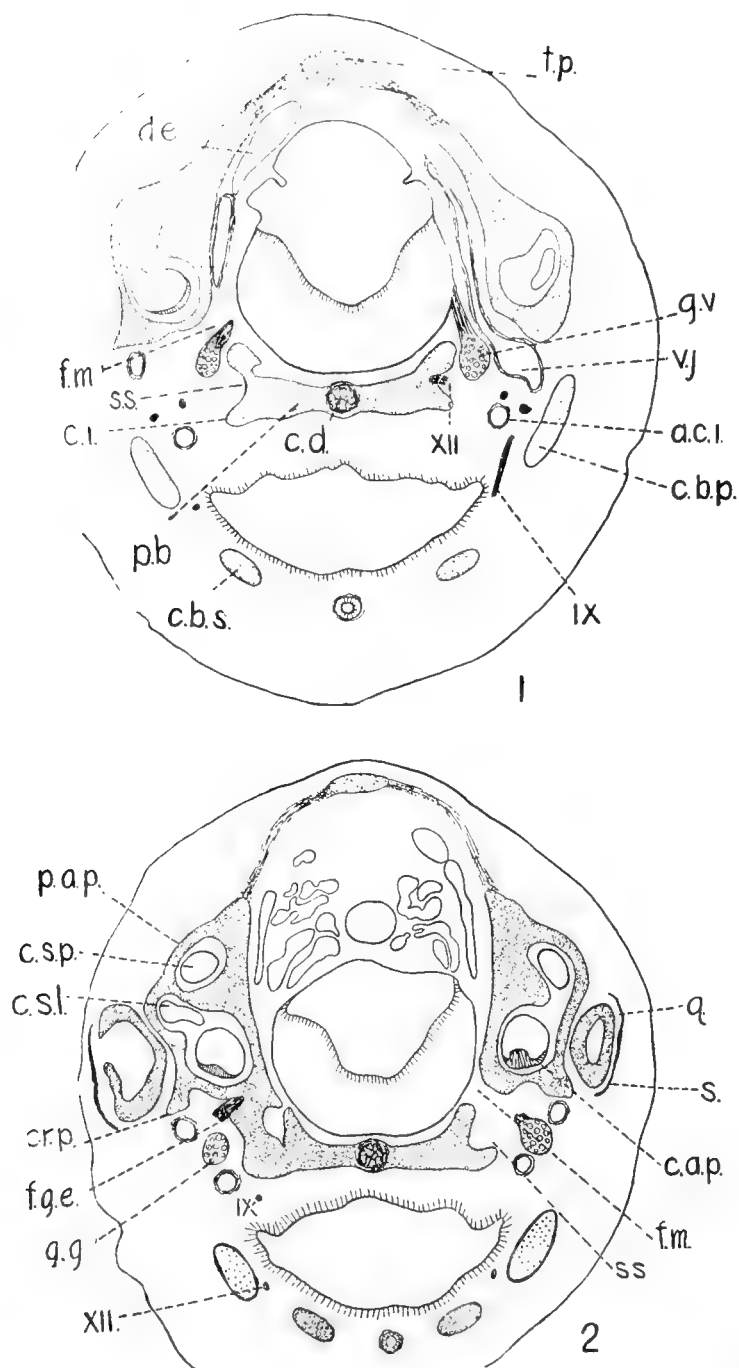


Fig. 1 Cross section through the posterior part of the otic region of an embryo having a carapace length of 11 mm. The section is slightly oblique, the left side (reader's) being anterior to the right side. $\times 15$.

Fig. 2 Cross section through the posterior part of the otic region of the same embryo as figure 1, and slightly anterior to it. On the right side the fissura metotica (*f.m.*) and sulcus supracristularis (*s.s.*) are shown and on the left side the canalis perilymphaticus opens into the cranial cavity by the extreme anterior end of the fissura metotica. $\times 15$.

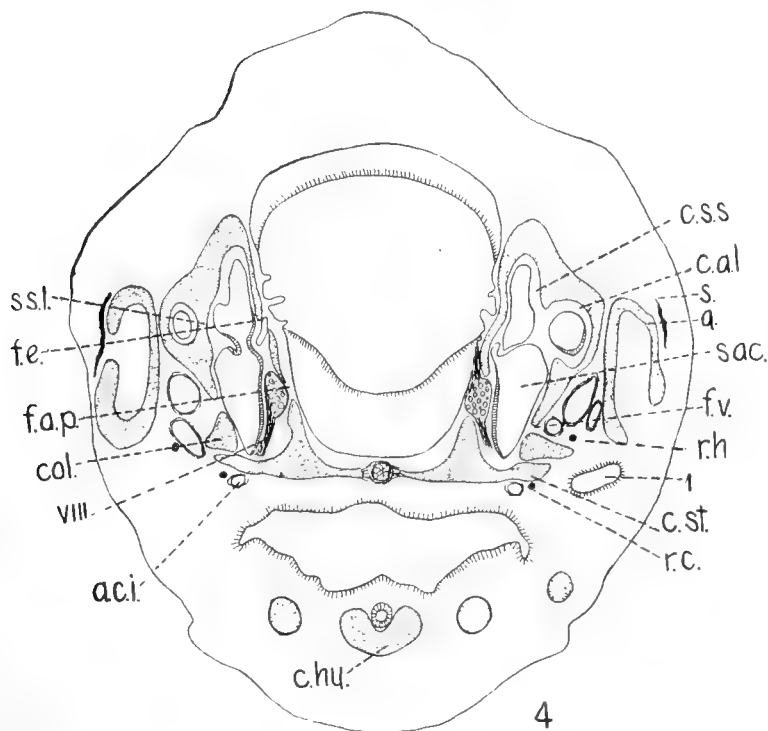
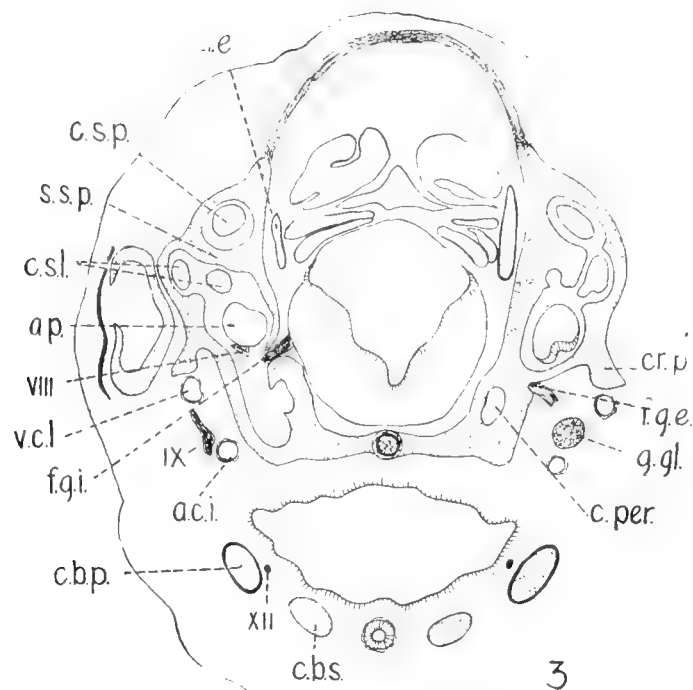


Fig. 3 Cross section through the otic region of the same embryo, showing the nervus glossopharyngeus on the right side emerging from the foramen glossopharyngei externum (*f.g.e.*) and on the left entering the otic capsule through the foramen glossopharyngei internum (*f.g.i.*). The canalis perilymphaticus (*c.per.*) is shown on the right side extending forward from the fissura metotica and on the left side it is opening into the cavum cochleae on the median side of the latter. $\times 15$.

Fig. 4 Cross section through the same embryo, slightly in front of the previous figure. The relation of the squamosum (*s.*) external to the quadratum (*q.*) is shown and the arteria carotis interna (*a.c.i.*) and ramus communicans n. facialis cum glossopharyngeo (*r.c.*) lying ventral to the crista substapedialis. $\times 15$.

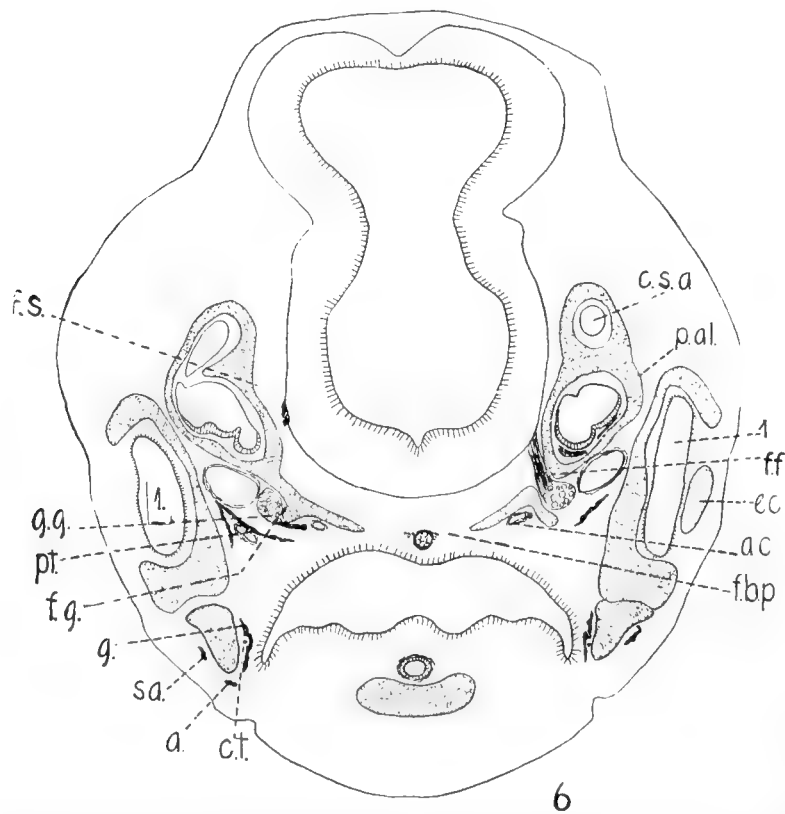
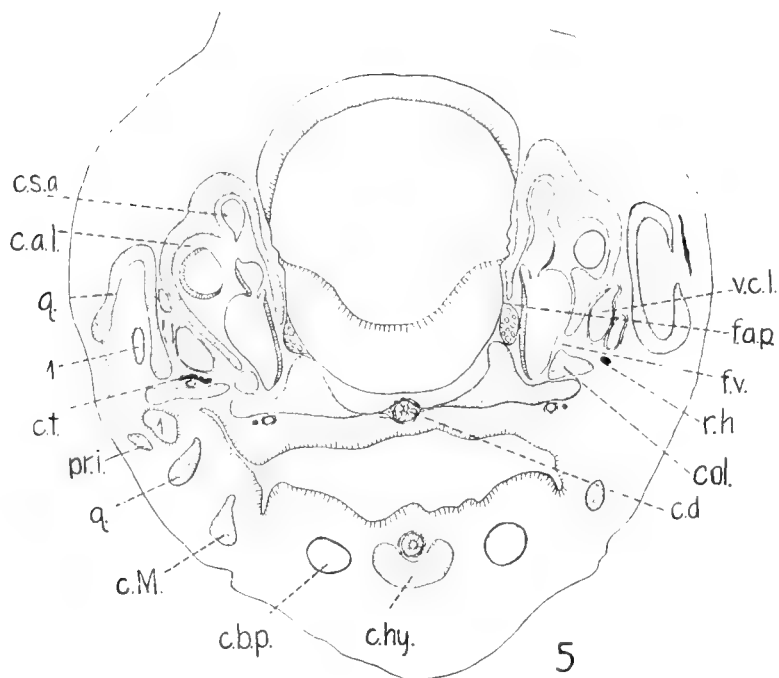


Fig. 5 Cross section of the same embryo, through the otic region, showing, on the left side, the chorda tympani (*c.t.*) passing from the n. facialis laterally on the dorsal side of the columella auris. The processus interhyalis (*pr.i.*) of the extracolumella is also seen. $\times 15$.

Fig. 6 Cross section of the same embryo through the anterior part of the otic capsule showing, on the right side, the foramen facialis (*f.f.*), and on the left side the fovea geniculi (*f.g.*) and the ramus palatinus n. facialis leaving the ganglion geniculi. The chorda tympani (*c.t.*) is lying in a groove on the lateral surface of the goniale. $\times 15$.

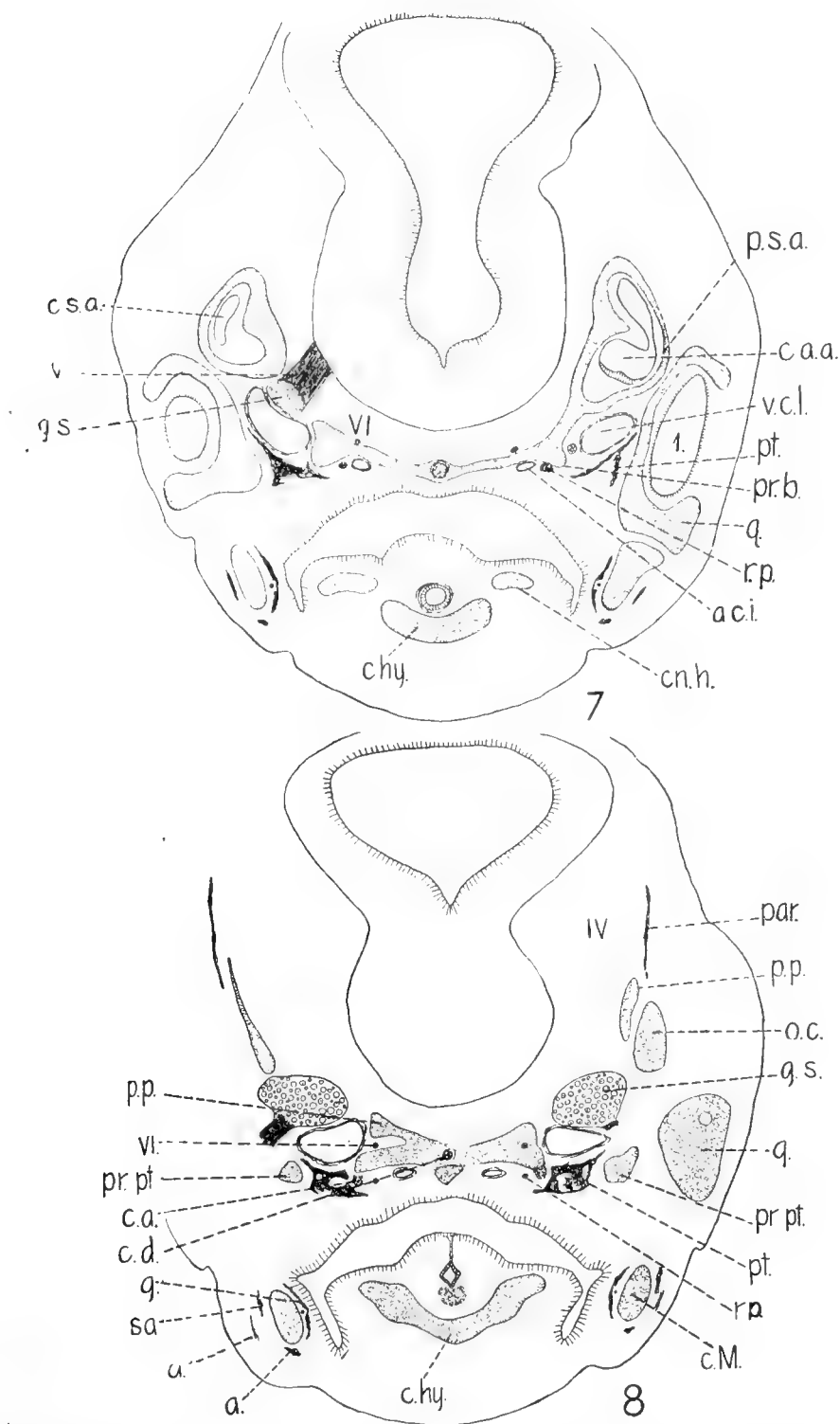


Fig. 7 Cross section of the same embryo through the anterior part of the otic region in front of the previous figure, showing the nervus abducens immediately before it enters the foramen abducentis and also the processus basiptygoideus (*pr.b.*). $\times 15$.

Fig. 8 Cross section of the same embryo through the posterior part of the orbito-temporal region, showing the large ganglion semilunare (*g.s.*) lying in the fenestra prootica; the nervus abducens (*VI*) is seen on the right side lying in the foramen abducentis and on the left it is ventral to the base of the pila prootica (*p.p.*). The cartilago articularis (*c.a.*) is to be seen attached to the ventral surface of the processus basiptygoideus. $\times 15$.

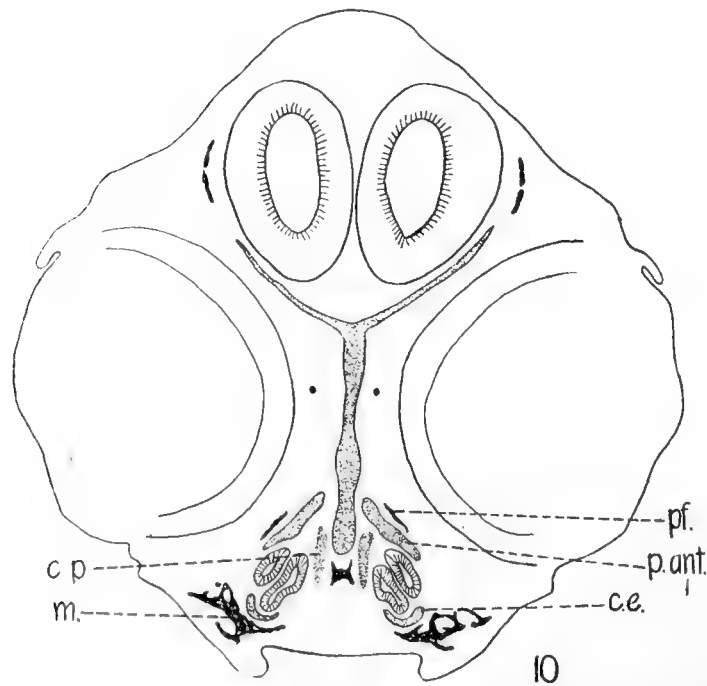
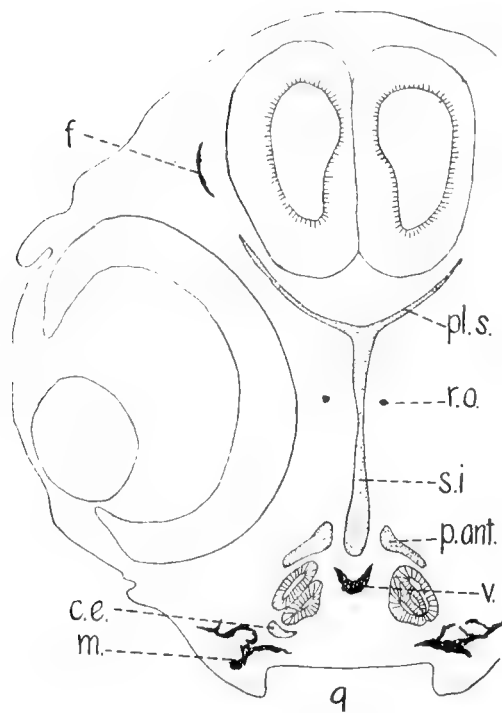


Fig. 9 Cross section through the anterior part of the orbital region of the same embryo, showing the septum interorbitale (*s.i.*) and the posterior part of the olfactory capsule, the planum antorbitale (*p.ant.*) and the cartilago ectochoanalis (*c.e.*) alone being cut. $\times 15$.

Fig. 10 Cross section through the posterior part of the ethmoidal region of the same embryo showing the lack of continuity between the olfactory capsule posteriorly and the septum interorbitale. $\times 15$.

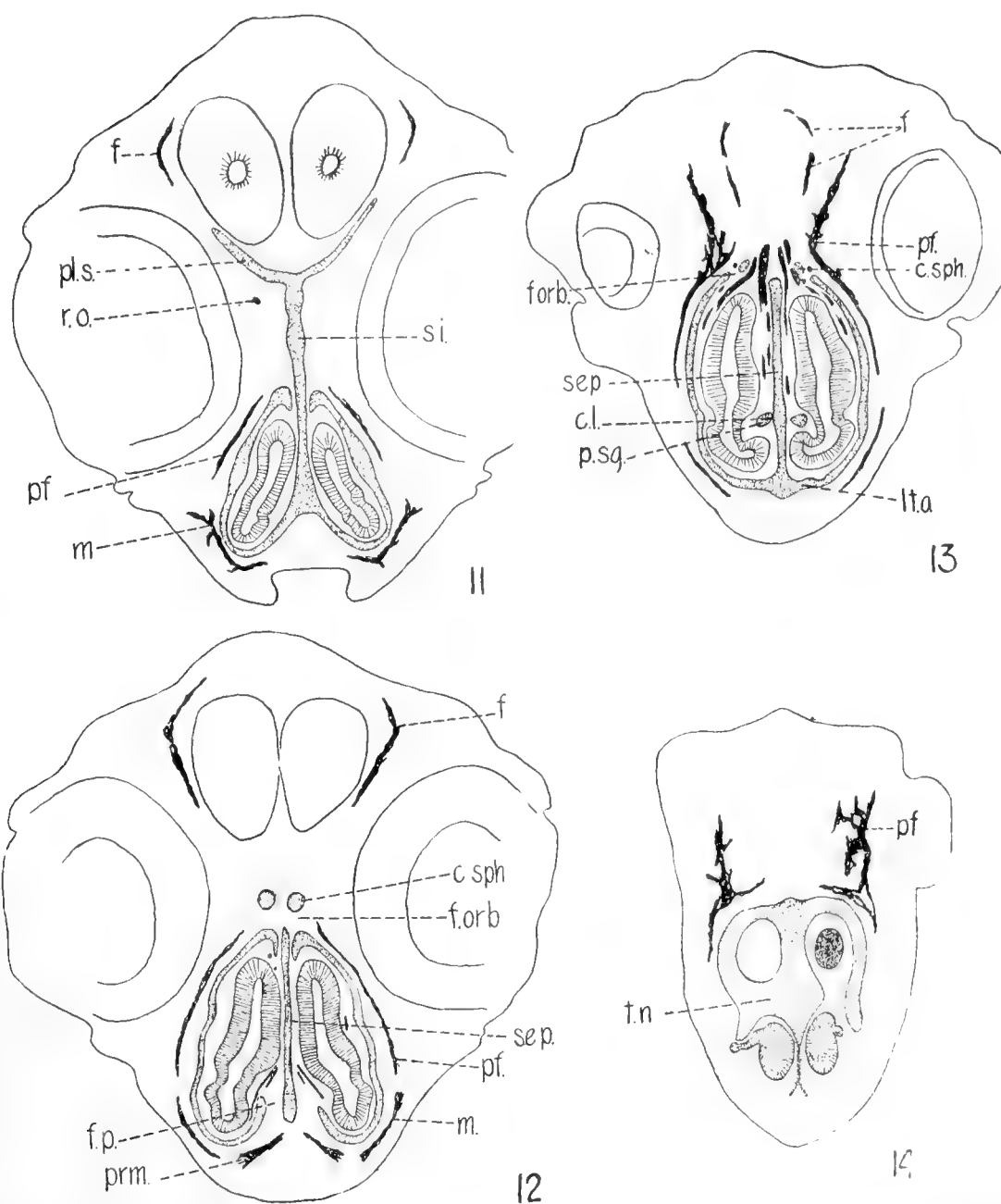


Fig. 11 Cross section through the ethmoid region a few sections in front of the preceding figure, showing the separation of the capsular wall dorsally from the septum, the thickening of the paries nasi to form a rudimentary concha, and the fusion of the planum nasi and cartilago paraseptalis with the septum posterior to the foramen praepalatinum which is seen in the following figure. $\times 15$.

Fig. 12 Cross section a short distance in front of the preceding figure, showing the foramen praepalatinum (*f.p.*) and the commissura sphenothmoidalis (*c.sph.*). $\times 15$.

Fig. 13 Cross section a short distance in front of the preceding figure showing the crista longitudinalis septi (*c.l.*), the pila supraglandularis (*p.sg.*), and the lamina terminalis anterior (*l.t.a.*). $\times 15$.

Fig. 14 Cross section through the extreme anterior end of the olfactory capsule of the same embryo, showing the glandula nasalis externa situated ventral to the cavity of the capsule. $\times 15$.

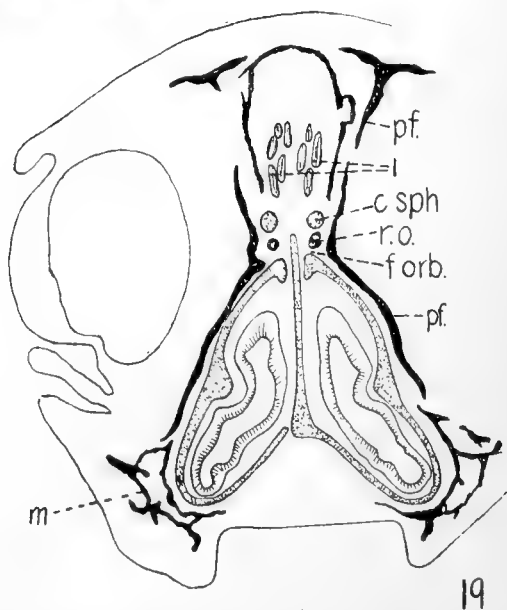
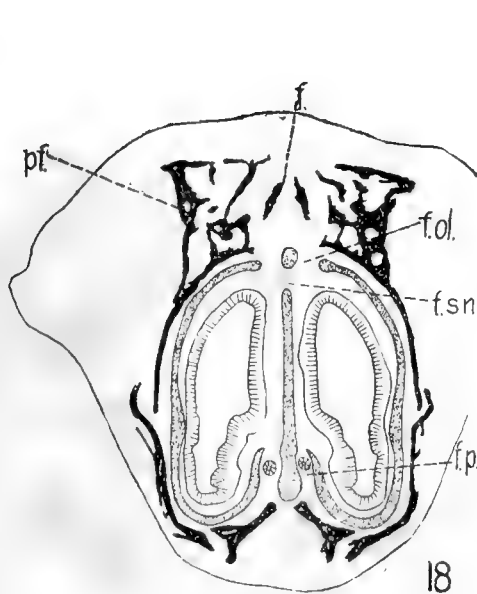
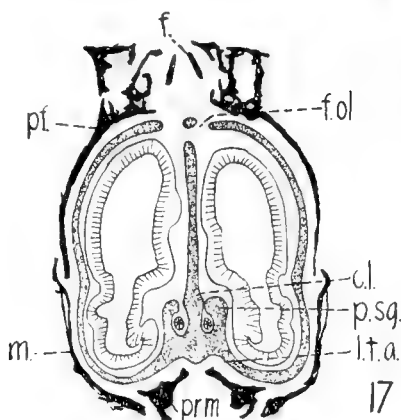
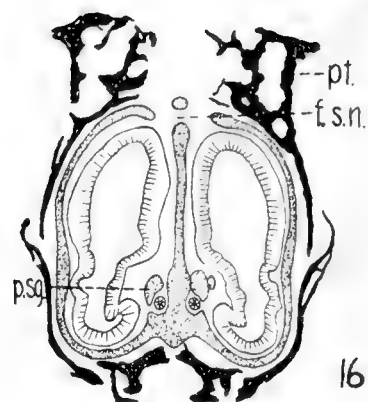
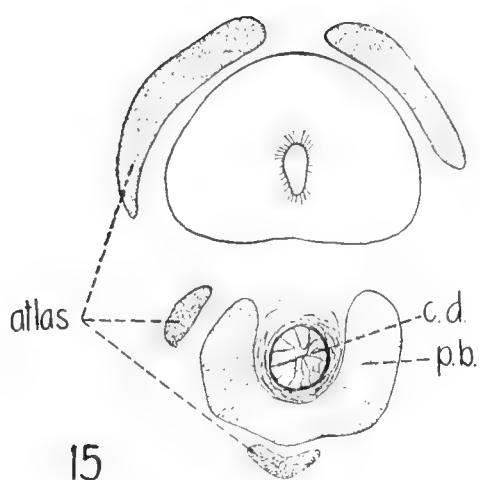


Fig. 15 Cross section through the extreme posterior end of the occipital condyle of an embryo slightly younger than that represented in the preceding figures, showing the chorda dorsalis (*c.d.*) incompletely surrounded by the occipital condyle which is here hypochordal in position. $\times 15$.

Figs. 16 to 19 A series of four cross sections through the ethmoidal region of an embryo having a carapace 13.5 mm. long. The series is from anterior to posterior. In figure 16, on the right side, a cartilaginous nodule is lying in contact by its median surface with the pila supraglandularis (*p.s.g.*). In figure 17 the pila supraglandularis has extended ventrally and fused with the lamina terminalis anterior (*l.t.a.*). The thickening of the paries nasi to form a concha is greatest in figure 19. $\times 15$.

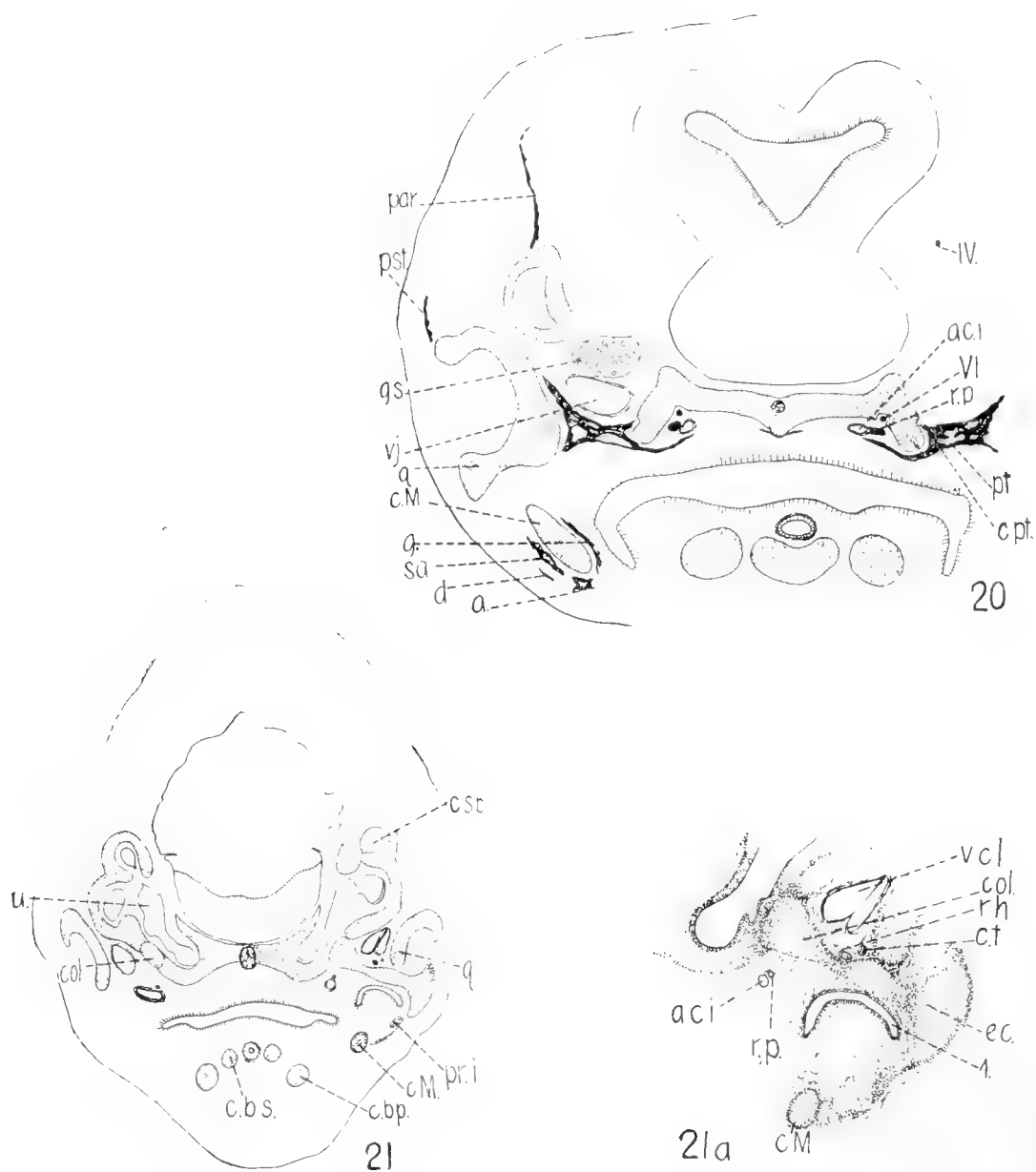


Fig. 20 Cross section through the posterior portion of the orbito-temporal region of an embryo having a carapace 13.5 mm. long, showing the pterygoideum (*pt.*) extending medially beneath the arteria carotis interna (*a.c.i.*) and the ramus palatinus n. facialis (*r.p.*) to form the sulcus cavernosus of the adult pterygoideum. A thin osseous lamella may also be seen along the ventral crest of the planum basale which extends further forward and fuses with the parasphenoid in the region of the fenestra hypophyseos. $\times 15$.

Fig. 21 Cross section through the otic region of an embryo having a carapace length of 7 mm. showing the columella auris (*col.*) in its relation to the first visceral cleft (*1*) to the ramus hyomandibularis n. facialis (*r.h.*), and to the chorda tympani (*c.t.*). The processus interhyalis (*pr.i.*) in its relation to the cartilago Meckelii (*c.M.*) is shown. $\times 15$.

Fig. 21 a A portion of figure 21 more highly magnified. $\times 35$.

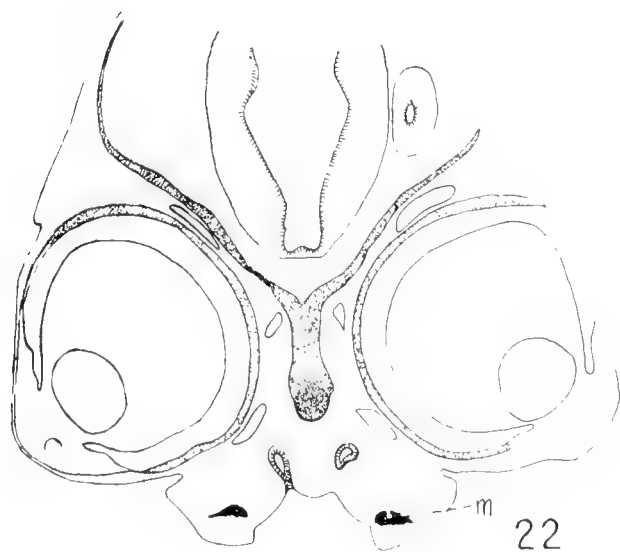
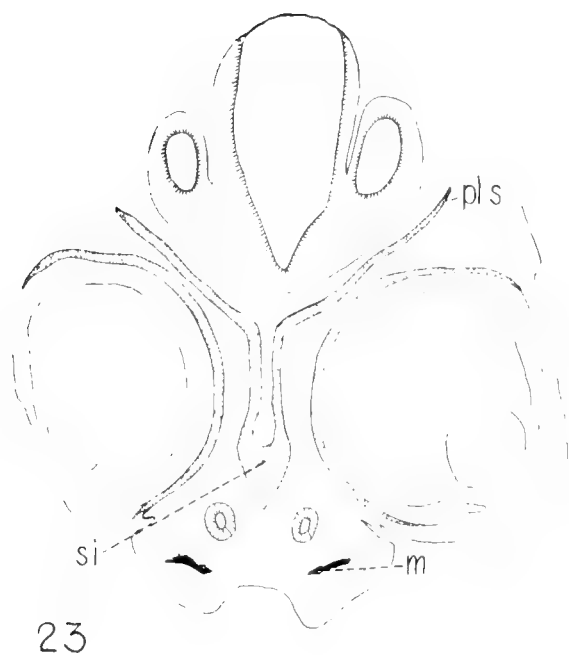
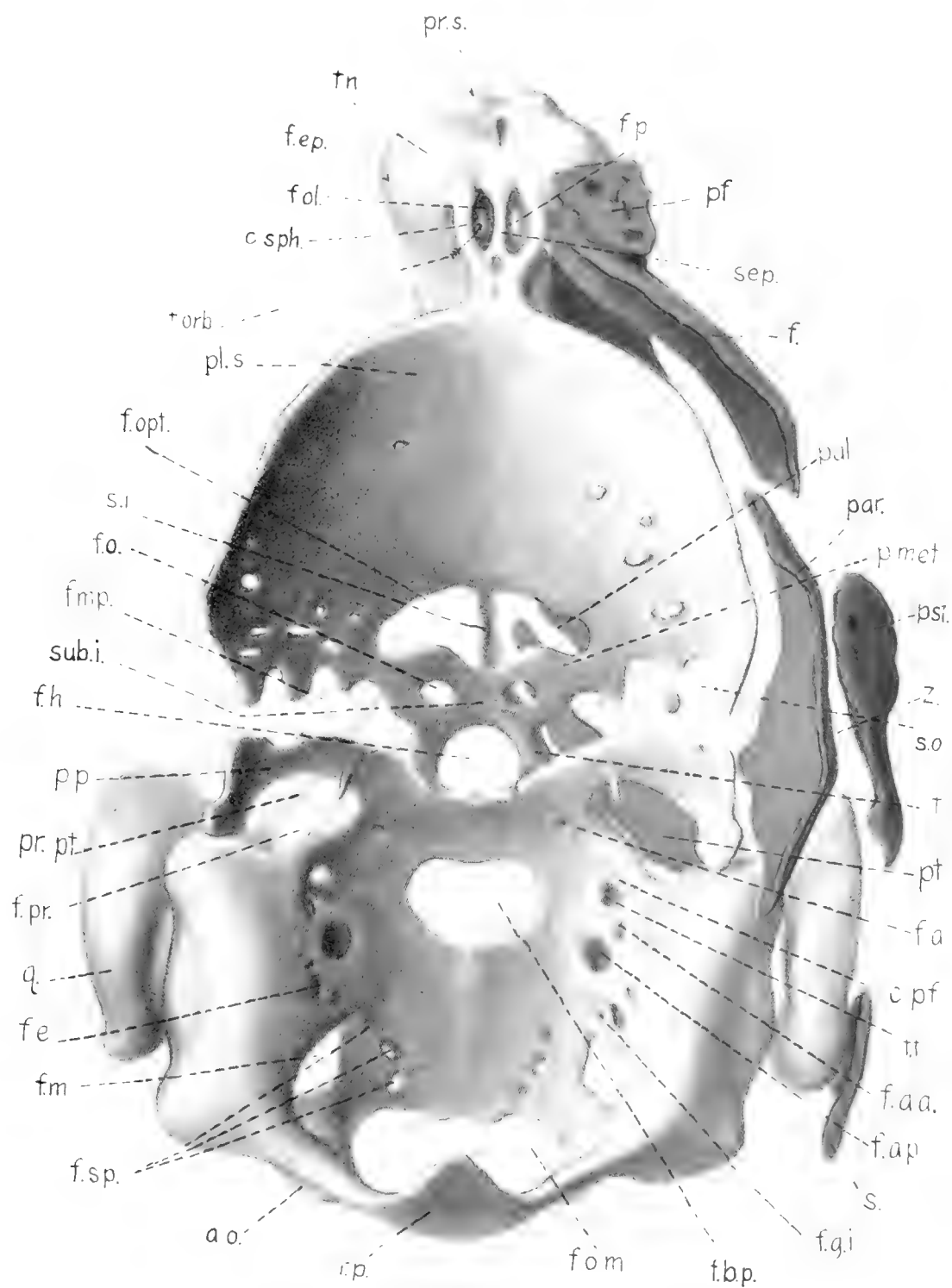


Fig. 22 Cross section through the anterior part of the orbito-temporal region of an embryo having a carapace 7 mm. long, showing the septum interorbitale much thicker from side to side than in an older embryo. $\times 15$.

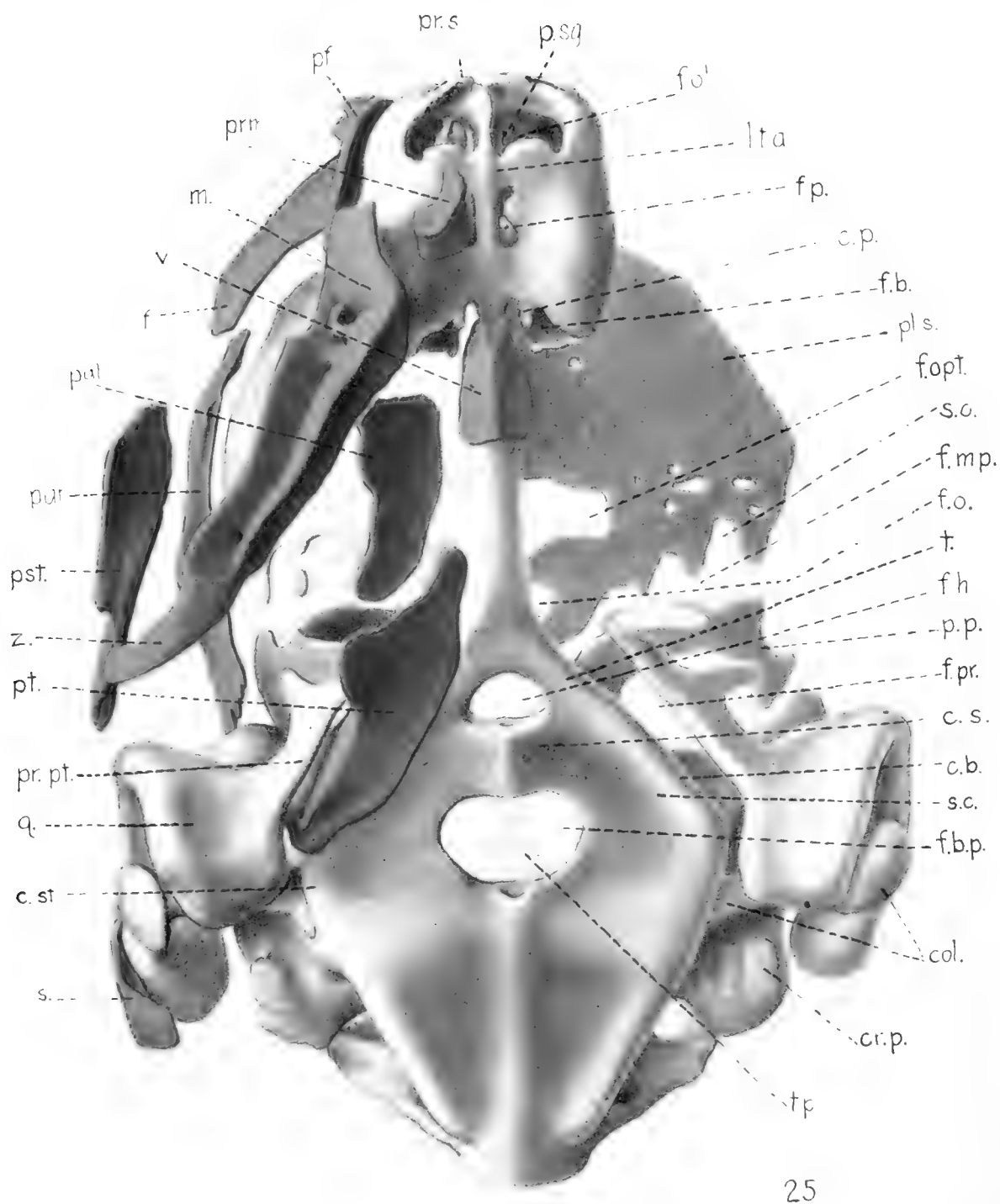
Fig. 23 Cross section through the same embryo as the preceding, somewhat further anterior than the previous figure, showing the septum interorbitale (s.i.) made up of two parallel plates which later become pressed together on the middle line. $\times 15$.



24

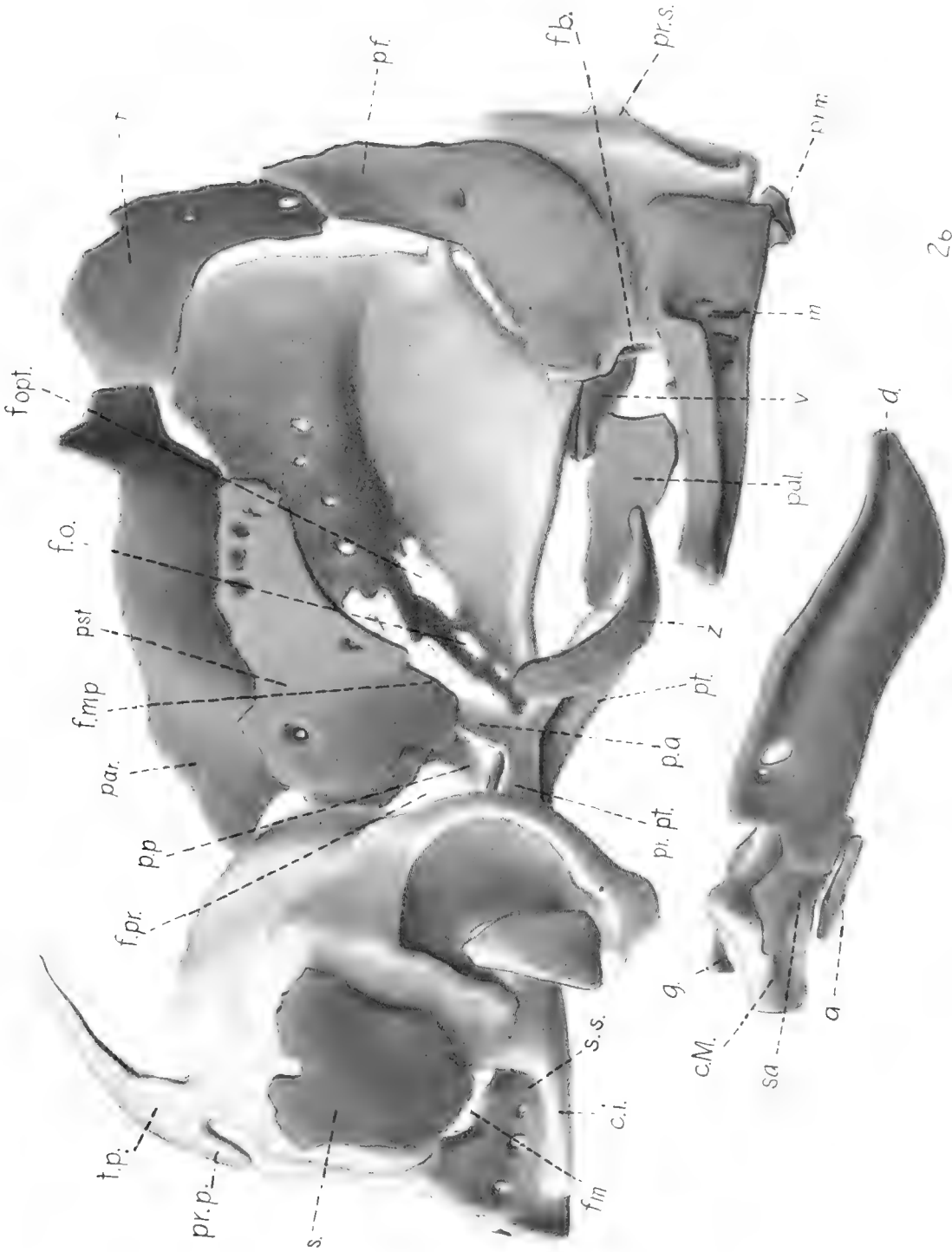
EXPLANATION OF FIGURE

24 Dorsal view of a model of the chondrocranium of an embryo having a carapace length of 11 mm. The membrane bones of the right side only are represented.
× 20



EXPLANATION OF FIGURE

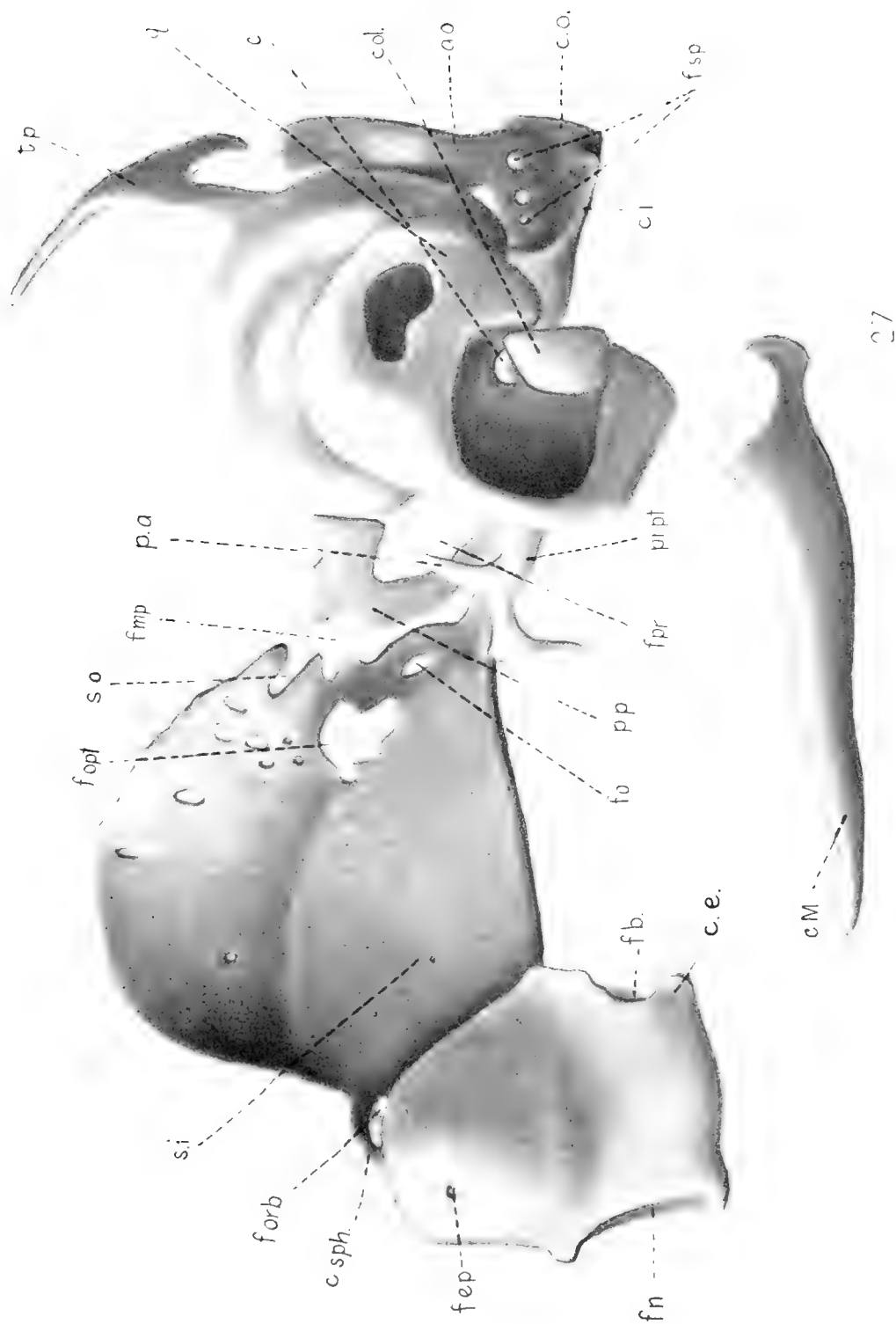
25 Ventral view of the same model. × 20



26

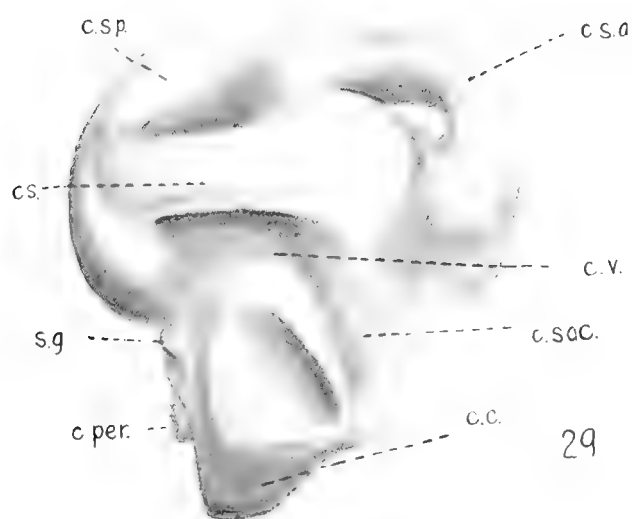
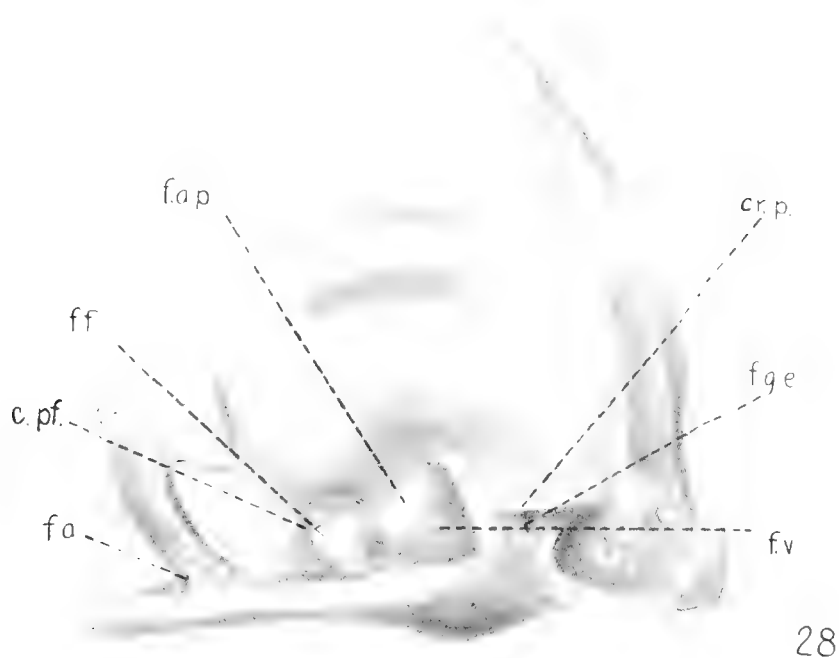
EXPLANATION OF FIGURE

26 View of the same model from the right side, showing also the mandible. $\times 20$.



EXPLANATION OF FIGURE

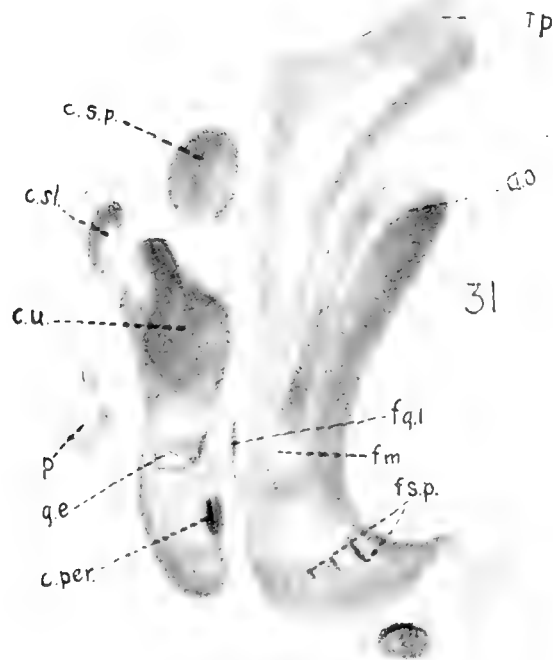
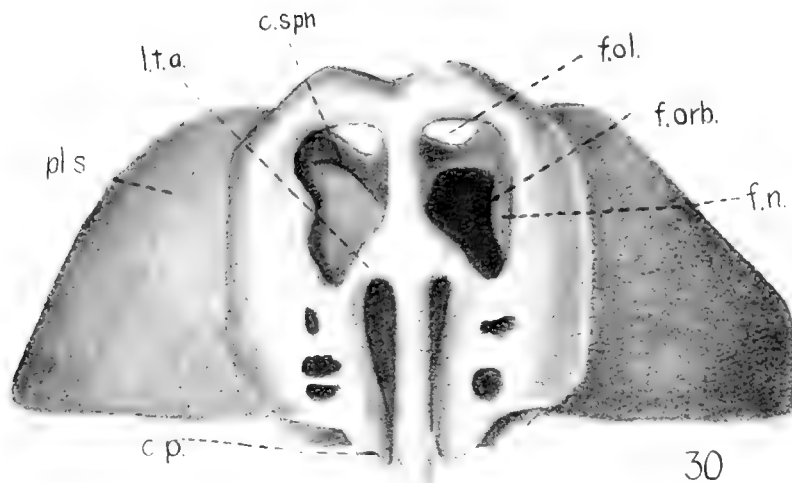
27 View of the same model from the left side. $\times 20$.



EXPLANATION OF FIGURES

28 View from left side of the same model showing the occipital and otic regions only, with the palatoquadratum removed in order to expose the lateral surface of the otic capsule. $\times 20$.

29 Lateral view of a model of the cavity of the right otic capsule of the same embryo as the above. $\times 24$.



EXPLANATION OF FIGURES

30 Ventral view of the olfactory capsule of an embryo having a carapace length of 7 mm. showing the cartilago paraseptalis (*c.p.*) separated from the septum nasi as far anterior as the lamina terminalis anterior (*l.t.a.*) and consequently the foramen praepalatatum not enclosed posteriorly. $\times 20$.

31 Posterior portion of the right otic capsule viewed from in front and slightly from the median line, showing especially the groove in which the n. glossopharyngeus passes through the capsule between the foramen glossopharyngei internum (*f.g.i.*) and the foramen glossopharyngei externum (*f.g.e.*), and also showing the opening of the canalis perilymphaticus (*c.per.*) into the cochlear cavity. $\times 24$.

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